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The Editor's Viewpoint 91

The Influence of Sodium Bisulphite on Acid Production in Saliva. *S. Dreizen, A. W. Mann, T. D. Spies, B. C. Carson and J. K. Cline* (Hillman Hospital, Birmingham, Alabama and Univ. of Cincinnati) 93

Dental Caries and Tryptophane Deficiency. *N. C. Turner and G. E. Crowell* (Forsyth Dental Infirmary) 99

A Note on the Duration of Caries Inhibition Produced by Fluoride Applications. *B. G. Bibby and S. S. Turesky* (Tufts College) 105

Solubility of Enamel as Affected by Fluorides of Varying pH. *R. W. Phillips and J. C. Muhler* (Indiana Univ.) 109

Solubility of Enamel Protected by Sodium Fluoride and Other Compounds. *J. C. Muhler and G. Van Huysen* (Indiana Univ.) 119

Studies in Dental Public Health Administration. II. The Role of Roentgenograms in Public Health Dental Surveys. *N. W. Chilton and L. E. Greenwald* (New Jersey State Department of Health) 129

Capacity of Periodontal Vessels on Pressure and Tension Sides of Teeth Moved by Orthodontic Appliances. *J. Revesz and S. W. Chase* (Western Reserve Univ.) 143

Experimental Amelotomy. *W. Lefkowitz, H. H. Shapiro and C. F. Bodecker* (New York) 151

Induced Fuso-Spirochetal Infection in Dogs. *H. M. Swenson and J. C. Muhler* (Indiana Univ.) 161

Bilateral Dens in Dente. *W. F. Swanson and F. M. McCarthy, Jr.* (Univ. of Pittsburgh) 167

International Association for Dental Research. Scientific Proceedings of the New York Section, January 30, 1947. *Frances Krasnow* (Guggenheim Dental Clinic) 173

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THE EDITOR'S VIEWPOINT

In a previous publication, Lefkowitz, Bodecker, Shapiro (*J. D. Res.* 23: 345, 1944) presented the results of removal of pulps from developing teeth. At that time they demonstrated an interference with the calcification of enamel as a result of removal of the pulp. In a further study (p. 151) the same authors have presented the results of the removal of the enamel organ of a tooth at about the same stage of development. In the present experiment, Lefkowitz, Bodecker, Shapiro have demonstrated that, in the absence of an enamel organ, the calcification of the enamel goes to completion, although enamel deposition is halted. They believe that this work, together with their earlier studies, demonstrates that the pulp is a factor in the calcification of enamel and that the enamel organ plays little or no rôle in the calcification of enamel after the matrix has been deposited. They are carrying out further studies on decalcified sections and are withholding definite conclusions until the publication of these results.

Swenson and Muhler (p. 161) induced fuso-spirochetal infection in dogs by the injection of scillarin B. The same drug had been previously used by Wallace, Wallace and Robertson to produce fuso-spirochetal infections and Tunnicliff and Hammond used it at the same time that they injected fuso-spirochetal organisms into guinea pigs to produce fuso-spirochetal lesions. The authors believe that there is a lowering of the tissue resistance which allows the organisms to enter the body. They were unable to confirm the finding of Rosenthal that sodium carbonate in the dog's saliva prevents formation of fuso-spirochetal lesions.

Turner and Crowell (p. 99) attempt to show a relationship between tryptophane deficiency and dental caries. In a previous paper (*J. D. Res.* 23: 413, 1944), Turner and Crowell gave data showing a correlation between caries experience and the amylase activity in saliva. Later, Berheim and Barnfield, (*J. D. Res.* 24: 141, 1945) were unable to demonstrate this correlation in another group of individuals. The authors, however, have studied the saliva of 7 individuals showing no caries and the saliva of 17 individuals showing rampant and active caries. The results indicate that there is a definite relationship between the tryptophane content of the saliva and the incidence of dental decay. They suggest that it may be possible to add tryptophane to the diet as a means of reducing dental caries. This gives another substance to add to the growing list of possible caries retarding agents.

Phillips and Muhler (p. 109) in a study of the solubility of enamel as affected by fluorides of varying pH present evidence that sodium fluoride is most effective at a pH of 2.6. The authors do not believe that this would be injurious to the tooth surface during the time ordinarily used for topical application.

Muhler and Van Huysen (p. 119) found stannic fluoride to be the most effective compound for use in reducing enamel solubility. If the value of fluorides is simply their reduction of the solubility of enamel, then such substances as stannic fluoride might be substituted for sodium fluoride in caries prophylaxis. It remains, however, to be determined whether sodium fluoride has some additional value when used in vitro, that is, whether or not its action is related solely to the reduction of enamel solubility.

Chilton and Greenwald (p. 129) evaluated the rôle of radiographs in public health dental surveys. As a result of their study they believe that dental x-ray examinations are not necessary to determine the dental needs of school populations, but that when surveys are conducted for research purposes radiographic examinations will be necessary for a proper interpretation of results.

Bibby and Turesky (p. 105) have exam-

ined the teeth of a group of 39 patients who had been treated over one quadrant of the mouth 6 times during the 2 year period with 0.1% sodium fluoride solution. The examination was made 3 years after the cessation of the treatment, and the treated quadrants showed approximately 36% less new decay than the untreated quadrants. The authors believe that the effect of sodium fluoride is of marked duration and present this result as evidence.

Dreizen, Mann, Spies, Carson and Cline (p. 93) noting that sodium bisulphite was used in the studies by Burrill, Calandra, Tilden and Fosdick on synthetic vitamin K activity in caries reduction (*J. D. Res.* 24: 273, 1945), studied the effect of sodium bisulphite alone. The dental caries activity was reduced in patients by the addition of sodium bisulphite in the form of a sodium bisulphite-containing paraffin troches. They believe that sodium bisulphite acts by interfering with the enzyme or co-enzyme systems involved in carbohydrate degradation process. In caries investigations it is very important that factors be singled out and that the caries-reducing result is not accredited to one factor when there may be others present.

The proceedings of the January 30th meeting of the New York Section of the International Association for Dental Research are given in abstract form. Five papers are presented on various subjects, including Psychomatic Factors in Periodontal Disease, Dietary Studies of Primative People, The Effect of the Temporal Muscle on the Form of the Mandible, Traumatic Injuries of the Children's Teeth, and Estrogenic Implants in Female Dogs.

A copy of the 8th Edition of the *National Formulary* has been received (Washington, D. C., American Pharmaceutical Association: 1946). In addition to the list of drugs listed in the National Formulary, the section on reagents and preparations for use in the clinical laboratory and on general tests, processes and apparatus should be of particular interest to those engaged in laboratory investigations and utilizing these various substances from time to time.

Dental Education Today (Harlan H. Horner (Chicago, University of Chicago Press, 1947) is a new addition to the small list of books on dental education. The first disappointment caused by the appearance that the book is only a compilation of the material of the Council on Dental Education's survey of the schools, together with numerous tables, is soon overcome when the reader becomes engaged by the discussion presented by Dr. Horner. There is a great deal of meat scattered through the many pages which discuss the backgrounds of dental education, the schools, faculty, students, curriculum, and teaching. The various organizations associated with dental education, including the International Association for Dental Research, are discussed. Dr. Horner, notes that the International Association for Dental Research has faithfully kept its promise to devote its interest to research. His mention of the Journal of Dental Research—"Abstracts of scientific papers are presented in the Journal of Dental Research, the official publication of the Association"—might lead one to the conclusion that the *Journal* is only an abstract publication and does not include original articles. The concentration of dental research work in a few schools is deplored by the author. He believes that the total absence of investigative attitude and habit in some schools, which does not depend merely upon material support is more disquieting than the lack of funds in other schools. The need for more research as a means of stimulating dental education is emphasized at several points. The discussion of the misunderstanding between the Council on Dental Education and the New York State Board on admission of students without all the predental requirements could be omitted without any loss to the spirit or content of the book. The book is one which will be carefully read by intelligent and interested dental teachers and by others who are interested in the future of dental education.

We have just received our December issue and here it is March, after many delays, some of them ours and some the printers. The blow that hurt was a letter from the printer dated January 30, 1947, to the effect that they were out of paper and the issue would be delayed until they received a new supply. Est-ce la guerre?

H. B. G. R.

THE INFLUENCE OF SODIUM BISULPHITE ON ACID PRODUCTION IN SALIVA[1]

SAMUEL DREIZEN, D.D.S., ARVIN W. MANN, D.D.S., TOM D. SPIES, M.D., BESS C. CARSON, A. B. AND J. K. CLINE, PH.D.[2]

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The rôle of acids in tooth decalcification (1) is now generally accepted. As a result recent attempts at dental caries control have been in the direction of decreasing the rate of acid formation in the mouth, and/or increasing the rate of acid neutralization in regions associated with the caries susceptible portions of the teeth (2). Since the latter has been considered difficult to achieve, methods and means of decreasing the rate of acid formation in the mouth have been the aim of most of the recent investigations in this field. Controlled diet, oral hygiene, and interference with the enzyme or coenzyme systems necessary for carbohydrate degradation have each presented distinct possibilities. Chemical inhibitors of enzyme systems has been studied by Stephan (3) using urea, Zander (4) using ammoniacal silver nitrate, Dean, Jay, Arnold, and Elvove (5) using the fluoride ion, and most recently Burrill, Calandra, Tilden, and Fosdick (6) using synthetic vitamin K. These investigators have all succeeded in reducing significantly the rate of acid formation in the saliva.

The work of Burrill, Calandra, Tilden, and Fosdick (6) was particularly interesting because a 2-methyl 1,4-napthoquinone-sodium bisulphite addition compound was employed. The results were attributed to the quinone which was regarded by these investigators as the active ingredient, the sodium bisulphite being used for the purpose of rendering the 2-methyl 1,4-napthoquinone water soluble so it would be more rapidly absorbed (7).

Sodium bisulphite, however, has had some use in medicine as an antiseptic and an antifermentation agent (8). The possible effect of sodium bisulphite on the results obtained by Burrill, Calandra, Tilden, and Fosdick stimulated investigation in regard to the action of this salt on the process of acid production in saliva. Since Burrill and his associates used less than 0.75 mg. sodium bisulphite in their study with 2-methyl 1,4-napthoquinone (6), a series of preliminary tests were made using varying concentrations of sodium bisulphite. It was found that values ranging from 2.5 mg. to 10 mg. showed a definite interference with the acid formation, the most efficient concentration ranging from

[1] These studies were supported by grants from Gelatin Products Company, and the American Dried Milk Institute. Received for publication December 2, 1946.

We wish to acknowledge the assitance of Miss Thelma Skinner in making the dental caries activity determinations.

[2] Associate Professor of Biochemistry, Medical College, University of Alabama, Birmingham, Alabama.

5.0 mg. to 7.5 mg., quantities much above those used by Burrill, Calandra, Tilden and Fosdick. No significant effect was observed with values below 1.0 mg. indicating, therefore, that the presence of sodium bisulphite in the form of an addition compound probably did not influence the results obtained by Burrill and his associates.

MATERIALS AND METHODS

Selection of Patients: Fifteen patients were selected from the personnel of the Nutrition Clinic, the Jefferson-Hillman Hospital of Birmingham, Alabama, and from patients referred to the Dental Department of the Nutrition Clinic by local practitioners. Each of these patients had been studied in the Clinic for the 2 preceding years and records of their dental caries activity during this period of time were available. The saliva of 11 of them had been consistently positive, 3 had been negative and one plus/minus. The patients, who appeared to be in good health, ranged in age from 13 years to 38 years, and included 13 females, one of whom was colored, and 2 white males.

Method of Study: Forty cc. of paraffin stimulated saliva were collected from each patient between the hours of 8:00 and 10:00 in the morning. Three different methods were used to test the saliva samples for dental caries activity. They were 1, the method of Fosdick, Hansen, and Epple (9), based on the difference in the amount of soluble calcium in saliva samples before and after incubation, 2, the method of Hadley (10), which determines the number of *Lactobacillus acidophilus* per cc. of saliva, and 3, the method of Dreizen, Mann, Cline, and Spies (11), which measures the buffer capacity of the saliva. One cc. of saliva was used for the bacteriological test, 5 cc. for the buffer capacity test, and 30 cc. for the calcium test.

The 30 cc. of saliva used for the calcium test were divided into 3 parts, each containing 10 cc. One part was immediately tested for calcium, the second part was incubated in the presence of tricalcium phosphate and dextrose for 4 hours at 37° C. in a shaker apparatus suspended in a water bath, and 7.5 mg. of crystalline sodium bisulphite were added to the third part which was also incubated under the same conditions. Following the incubation period the 2 samples of saliva so treated were tested for soluble calcium by titrating against 0.01 N $KMnO_4$ using the method of Fosdick, Hansen, and Epple (9).

To determine the effect of the salt in saliva treated in vivo, 7.5 mg. of crystalline sodium bisulphite were incorporated in a paraffin block. The patients were permitted to rest from ½ to 1 hour after the collection of the first sample, following which 30 cc. of saliva were again collected, using the bisulphite-containing paraffin block as a secretory stimulant. The subjects were cautioned not to swallow any of this saliva during the period of collection. The 3 different laboratory tests for caries activity were then repeated on the saliva thus obtained.

OBSERVATIONS

No significant differences were observed in the buffering capacity of the saliva in any of the 15 patients when the saliva samples containing sodium bisulphite

were compared with those free of bisulphite. Neither were there any appreciable differences in the lactobacillus counts in the bisulphite-treated and untreated salivas.

The results obtained with the chemical tests, however, showed a striking difference. As may be seen from Table I, the production of acid in the saliva was interfered with in every case in which sodium bisulphite was added. This phenomenon was also manifested in the salivas collected by stimulation with the sodium bisulphite-containing paraffin blocks. Regardless of the dental caries activity of the untreated saliva, all but one of the sodium bisulphite samples gave a negative reaction when tested. In the one instance in which a

TABLE I

*Results of chemical test for dental caries activity on untreated saliva and saliva treated with sodium bisulphite in fifteen patients**

PATIENT	UNTREATED SALIVA		SALIVA TREATED IN VITRO†		SALIVA TREATED IN VIVO‡	
	Activity	pH reading after incubation	Activity	pH reading after incubation	Activity	pH reading after incubation
1. T. J. McK.	++++	4.55	−	6.61	−	6.75
2. E. R.	+++	4.71	−	7.00	±	6.55
3. E. S.	+++	4.61	−	6.59	−	6.15
4. E. M.	+++	4.48	−	6.39	−	6.40
5. B. C. C.	+++	4.88	−	6.81	−	6.00
6. T. A. S.	++	5.61	−	7.02	−	7.12
7. M. H.	++	4.51	−	6.30	−	6.70
8. J. C.	++	5.06	−	7.06	−	6.80
9. L. M.	++	5.15	−	7.31	−	6.02
10. A. B. H.	++	4.88	−	6.90	−	6.63
11. S. W.	+	4.95	−	6.90	−	6.00
12. M. F. H.	±	5.82	−	6.90	−	7.08
13. A. L. B.	−	6.51	−	7.00	−	6.89
14. S. D.	−	6.77	−	7.30	−	7.09
15. C. B.	−	6.08	−	6.91	−	6.81

* The chemical test used is that of Fosdick, Hansen, and Epple.

† Saliva samples to which 7.5 mg. sodium bisulphite were added directly.

‡ Saliva stimulated by paraffin containing 7.5 mg. sodium bisulphite.

plus/minus value was obtained, the patient admitted having swallowed some of the sodium bisulphite despite advance instructions not to start mastication of the paraffin block until it had been sufficiently softened by the warmth of the mouth.

To emphasize further the influence of the sodium bisulphite upon the acid production of the saliva of these patients, pH readings were taken of the saliva samples removed from the water bath at the end of the 4-hour period of incubation. A Beckman pH Meter was used to make the determinations. These results are also summarized in Table I where it is noted that in every instance the untreated salivas had a lower pH reading than the bisulphite-treated specimens of the same saliva.

DISCUSSION

The findings obtained with the chemical test for caries activity indicate that the sodium bisulphite exhibited an inhibitory effect on acid production in the saliva at levels above 1.0 mg. In attempting to explain the mechanism of the action of sodium bisulphite, it is obvious that the reduced acid production could not be attributed to dilution as the salt was added in the crystalline form. The action was not a neutralization as the salt is normally acid in reaction, 7.5 mg. dissolved in 10 cc. of distilled water having the pH of 5.1. As the addition of the sodium bisulphite did not change the buffer capacity of the saliva, the decreased acid production could not be accounted for on this basis. The fact that the bacteria persisted in their growth in the presence of sodium bisulphite, indicates that the action is not a germicidal one. Curtis and Scott (12) point out that sulphites and hyposulphites, while maintaining their chemical composition as such, are practically devoid of either germicidal or antiseptic properties. The possibility of the sulphite and bisulphite ions drawing calcium out of solution and thereby producing "false" negatives was investigated, but did not occur with the concentrations used in this study.

The decreased acid production can, however, be interpreted as resulting from an interference with the carbohydrate degradation system in which the *Lactobacillus acidophilus* and the other acidogenic organisms present in the mouth take part. Sodium bisulphite is a reducing agent (8), and this property may conceivably account for the reduced acid formation in the saliva since reducing agents are classified as inhibitors of enzyme action (13). Sulphites,[3] even in minute amounts, are also known to interfere with the action of ferments. (14) They modify the process of fermentation in part by uniting with aldehydes which interferes to a marked degree with the aldehyde serving as a hydrogen acceptor in the development of pyruvic acid, the hydrogen going instead to the formation of glycerol (15). The fixation of glyceraldehyde, which is produced in the primary decomposition of the glucose molecule, would of necessity interfere with the acid production in saliva since the glyceraldehyde is believed to be modified to methyl glyoxal hydrate, the mother substance for all subsequent changes leading to the formation of lactic acid (16). Should interference with co-carboxylase alone have occurred as might have been expected by sulphite splitting of the thiamin moiety, increased acid production instead of the observed decrease would have been the result. These findings can in no way be interpreted as demonstrating any clinical usefulness of this product. Possible clinical applications are now under consideration.

SUMMARY AND CONCLUSIONS

1. The dental caries activity of untreated saliva and of saliva treated with sodium bisulphite of 15 patients was determined by 3 different laboratory tests.

[3] The significance of the sulphite radical was demonstrated when sodium pyrosulphite was substituted for sodium bisulphite in 7 cases. All cases positive without the pyrosulphite were rendered negative (Fosdick, Hansen, and Epple) with the addition of pyrosulphite.

2. The addition of sodium bisulphite produced a marked decrease in the acid production of the saliva of each patient.

3. The sodium bisulphite apparently acts by interfering with the enzyme or coenzyme systems involved in carbohydrate degradation process. The sulphite radical combines with the aldehydes formed in the breakdown of carbohydrates and prevents the formation of lactic acid.

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DENTAL CARIES AND TRYPTOPHANE DEFICIENCY[1]

NAOMI C. TURNER, Ed. M., and GEORGE E. CROWELL, D.M.D.*

A correlation between caries susceptibility and an enzymic factor in the saliva has been reported by Turner and Crane (1). An extention of these studies reveals something more of the nature of the correlative factor. A lack of tryptophane or similar indole containing molecule[2] shows a striking correlation with the degree of dental decay.

The saliva of 7 individuals showing no caries produced a purple coloration upon testing with glyoxylic acid (pH 4.5–6.5) and concentrated H_2SO_4. The saliva of 17 individuals showing rampant and active caries was tested. Sixteen of these gave a nearly negative or trace reaction, with only a very faint narrow lavender line. Upon running the final ½ cc. H_2SO_4 through the saliva-glyoxylic acid layer this line diffused to a light lavender or pink, in some cases. One individual with extensive caries showed ¼ inch lavender. In 6 of the no-caries associated salivas from ¾ inch purple over green to 1 inch of purple color developed. The saliva of 1 individual with no caries showed ¼ inch purple coloration. That we are measuring present saliva state against future tooth conditions must be borne in mind.

The following modification of the method of Shaw and MacFarlane[2] was used for the estimation of tryptophane. To 1 cc. saliva in a graduated test tube 1 cc. glyoxylic acid was added. After gentle but thorough mixing, 1½ cc. concentrated H_2SO_4 was introduced down the side of the tube. After allowing a minute for the development of the color ring at the juncture of the two layers ½ cc. of concentrated H_2SO_4 was dropped cautiously through the mixture to determine whether additional color would develop.

A further differentiating test for salivas was devised. The time to take the color out of preformed starch blue, pH 6.8, redox (reduction-oxidation potential) + 322 mv, was used as a measure of reductive activity of the saliva. That reductive activity in saliva is enzymic rather than bacterial was pointed out by Pincus (3). For the individuals with no caries, the time average for decolorization of preformed starch blue was 4.8 minutes. For the individuals with active rampant caries, the average time was 13 minutes. If further experimentation bears out these findings, the simplicity of this test as contrasted with the tedious and exacting methods we have previously reported would indicate much greater practicability for use in dental offices.

[1] Received for publication January 11, 1947.

* Facilities for clinical studies were made available through the courtesy of the Forsythe Dental Infirmary, Boston, Massachusetts.

[2] Since tryptamine as well as tryptophane condenses with aldehydes at ordinary temperatures and at pH 5–7 to give a color ring, the possibility exists that the effective saliva form may be tryptamine rather than tryptophane.

The individual salivas from these 2 groups was studied on the Beckman potentiometer with calomel and platinum electrodes. Tests were made on whole specimens of saliva with the realization that the saliva in immediate contact with the carious process might vary somewhat in nature. The average

TABLE I

INDIVIDUAL	TRYPTOPHANE TEST	DEXTRINIZING TIME	DECOLORIZATION TIME	REDUCTION-OXIDATION POTENTIALS				
				Initial redox	10 min. reading	Poise change 10 min.	30 min. reading	Poise change 30 min.
Extensive caries								
		min.	min.					
E. Bu.	trace	20	13	+202	−621	823	−642	844
M. C.	negative	6	—	+323	+239	84	+100	223
J. R.	¼ in. lav.	10	—	+320	+172	148	000	320
R. D.	trace	10	—	+308	+170	138	−180	488
J. O'K	trace	15	—	+320	+002	318	−210	530
H. B.	trace	5	—	+330	+192	138	+029	301
E. L.	trace	10	12	+360	+210	150	000	360
K. L.	¼ in. lav.	15	10	+320	+081	239	−410	730
L. N.	trace	11	9	+365	−050	415	−531	896
B. D.	trace	10	9	+320	+215	105	−020	340
J. A.	trace	10	9	+318	−110	428	−406	724
O. R.	trace	15	—	+208	+098	110	−415	623
F. G.	trace +	10	14	+300	−402	702	−606	906
M. C.	¼ in lav.	20	10	+250	+068	182	−560	810
P. T.	trace	20	15	+315	+115	200	−240	555
J. S.	trace	15	21	+340	+182	158	−350	690
L. W.	¼ in. lav.	20	21	+355	+240	115	000	355
Average.....		13	13	+309		261.9		570.3
Caries free								
E. Bo.	1 inch faint lav.	90	5	+279	+185	94	−021	300
E. W.	1 inch faint purple	50	4	+276	+238	38	+158	118
T. L.	¼ in. purple	78	6	+230	−265	495	−396	626
J. H.	¾ in. green and purple	90	6	+340	−202	542	−504	844
D. M.	1 in. purple	75	3	+350	+224	126	+134	216
R. M.	1 in. light purple	60	4	+330	+240	90	+200	130
M. N.	¾ inch purple br.	45	6	+350	+250	100	+160	190
Average.....		69.7	4.8	+307.9		212.1		346.3

10 minute redox poise drop for the carious group was 261.9 mv from the positive toward the negative.[3] The 10 minute redox poise drop for the caries-free group was 212.1 mv. At 30 minutes the difference had widened. The 30 minute redox

[3] The Lewis and Randall convention is used in the interpretation of positive and negative redox numbers.

poise drop for the caries susceptible group was 570.3 mv. The 30 minute redox poise drop for the caries free group was 346.3 mv. Within each group the variations in redox figures for the individuals is so great that it is obvious, as one might expect, that other factors than amylase activity affect redox poise of saliva. In our experience some of these factors are fatigue, excitement, and virus infection. Hence diagnostically and prognostically the potentiometer at this time cannot be used as an indicator of caries susceptibility. However, the average of the caries-free group's drop was slower toward the oxidative side than the average of the carious group's drop.

An experimental dosage of d-1 tryptophane adjusted to body weight was ingested by a group of young adults on 4 successive days. The material was taken in crystalline powder form with as much water as desired. The ingestions were midway between breakfast and lunch. Previous ingestion in gelatin capsules had proven less effective in producing salivary changes.

The ingestion of tryptophane by these adults yielded the following changes: The time required for the saliva to reduce preformed starch blue was changed from 15 minutes on the average to 6 minutes on the average. The controls did not decrease their salivary reducing time. Reduction-oxidation potentials for 6 members of the group were taken before and after tryptophane ingestion. The Beckman potentiometer was used with the calomel and platinum electrodes. Redox poise may have been altered by the ingestions. The average 10 minute redox poise drop before tryptophane for this young adult group was 132 mv. After ingestion it was 111 mv. It is notable that this is in the same direction as the no-caries saliva shows in relation to the carious saliva. Two individuals failed to follow the group tendency at 10 minutes. Each had been ingesting niacin. One took 20 mg. daily in a complement of vitamins. The other had taken 450 mg. niacin in the week prior to testing. Among unpublished material we have some evidence that the vitamin B complex or some members of the vitamin B complex speed dextrinising time.

There was not a significant change in body weight during this period; nor a consistent change in blood pressure. In 5 individuals the presence of tryptophane was determined in the blood serum as well as in the saliva. There was a notable parallel in the amounts in the saliva and in the blood serum for each individual whether the tryptophane occurred naturally or followed tryptophane ingestion.

The change in fasting blood sugar would appear to be significant.

	Individual Blood Sugar Readings	
	Before Tryptophane	*After Tryptophane*
R. C.	91 mg.%	72 mg.%
C. M.	75	67
P. H.	87	89
N. T.	113	63
L. W.	87	71
B. V.	96	64
S. T.	106	88
N. B.	101	66
O. F.	101	67

		Comparable Readings for Controls Who Did Not Ingest Tryptophane
J. W.	91	84
L. K.	71	84

Among the 9 individuals in this series ingesting tryptophane only 1 failed to show a decrease in blood sugar. This individual changed from 87 mg.% to 89 mg.%. For the other 8 the average fasting blood sugar was 96 mg.% (range 75–113). After the tryptophane ingestions the average was 70 mg.% (range 63–88). This drop of 26 points on the average may be of interest in diabetes and possibly in hypoglycemia. For the present when tryptophane is given experimentally, blood sugars should be watched. The average of the blood sugar of the controls did not drop.

Correlation between active caries and a rapid hydrolyzing (dextrinizing, amyloclastic) time (1), using the starch, KI, H_2O_2 procedure (4) was again demonstrated; as was also correlation between freedom from caries, and a slow hydrolyzing time.

Active caries average time—13 minutes (range 5–20 min.)

No caries average time—69.7 minutes (range 45–90 min.)

Some laboratories have found difficulty in establishing this relationship. Among the causes for failure of the demonstration have been found to be: (a) use of stimulated saliva, (b) failure to take saliva at least 2 hours after a meal, i.e., use of non-resting saliva, (c) use of saliva which has stood between drawing and testing, (d) use of saliva following smoking, (e) use of a temperature higher than 20° during testing procedure, (f) use of soluble starch, (g) use of citrate buffered starch. In other cases failure to note the relationship was due to lack of differentiation between the amyloclastic and the saccharogenic phase of amylase activity. A diastase method which does not differentiate the 2 phases would not in our experience be expected to show a correlation with caries.

Certain facts from the work of others gives additional meaning to this relationship of fast dextrinizing time associated with severe and active caries versus slow dextrinizing time associated with freedom from caries. Schwimmer (5) has shown dextrinizing time to be a function of α amylase. Kneen (6) has shown the calcium ion to be a stability factor for α amylase and to be a deterrent to β amylase. Purr (7) reports the occurrence of a pancreatic β amylase (which was activated by vitamin C). Fosdick (8) has shown that the saliva of carious individuals withdraws calcium faster than the saliva of caries-free individuals. The salivas of caries immune individuals was compared with the salivas of caries susceptible individuals; it was found that the latter withdrew on the average 11.1 mg. of calcium from 4 grams powdered human tooth enamel after 4 hours agitation at body temperature; whereas the saliva of caries immune individuals withdrew no calcium.

Saliva from mouths with active dental decay has a high concentration or proportion of α amylase (the dextrinizing enzyme) (1). Calcium is required for maximum α amylase activity (6), and these salivas show a high capacity for withdrawal of calcium (8). The saliva of caries free individuals has less dextrinizing ability, and may have β amylase in higher proportion to α. β amylase

is deterred by the calcium ion and salivas from caries-free mouths have been shown by Fosdick not to withdraw calcium from powdered human tooth enamel. These facts, together with the redox findings and preformed blue decolorization times, force us to the conclusion that caries associated saliva is more oxidative in its action on starch while caries-free saliva is more reductive. A highly oxidative enzyme and a more reductive enzyme would be expected to act on starch quite differently. Assuming an aldehyde at the end of the starch molecule, oxidation would lead to acidic products, while reduction would lead toward products like alcohol.

$$R{-}C(=O){-}H \xrightarrow{\text{oxidation}} R{-}COOH$$

$$R{-}C(=O){-}H \xrightarrow{\text{reduction}} R{-}CH_2OH$$

While it has not been demonstrated that saliva acting on starch creates oxalic acid, it is known that (in vitro) oxalic acid is formed from starch by oxidation at 37° C (9). An early technical method of preparation of oxalic acid was from cellulose (10). Molds acting on sugar yield a high proportion of oxalic acid if pH 6–7 is maintained by the addition of calcium carbonate. Oxalic acid is one of the strongest organic acids and is of acidic strength comparable to that of a mineral acid. Oxalic acid is both in the laboratory and in the living organism noted for its capacity to precipitate calcium. Thus if saliva permits the production of oxalic acid from starch, the resulting calcium withdrawal may in part account for the carious disintegration of tooth substance. The saliva of caries-free individuals may accomplish starch breakdown without interference with calcium metabolism.

The presence of the amino acid tryptophane seems to increase the enzyme's reductive reactions towards starch, and thus may help to prevent calcium withdrawal. By glyoxylic acid test β amylase shows a tryptophane (or tryptophane-like) purple ring. This, together with the fact that caries-free salivas show high tryptophane (or tryptophane-like) content and slow dextrinizing time leads to the hypothesis that these salivas are not only relatively low in α amylase but also may contain β amylase. A significant point subject to measurement is the 2-minute redox poise drop of[4] α and β amylase. In 2 minutes under the calomel and platinum electrodes α amylase dropped 36 mv while β dropped only 6. It is reported above that saliva from mouths with dental decay drops on the average more rapidly than saliva from mouths free from decay, and that the ingestion of tryptophane slows the drop. This further strengthens the theory that saliva from mouths with dental caries is disproportionately high in α amylase. Since β amylase activity is favored at pH 3 (6) we may have a further explanation of why β amylase activity on starch does not adversely affect teeth. Since the addition of tryptophane to alpha amylase prolongs the dextrinizing time of alpha amylase working on starch, one must consider the possibility that tryptophane acts as an inhibitor with this enzyme.

[4] α and β obtained from Wallerstein.

SUMMARY AND CONCLUSIONS

1. Four tests have shown a relationship between the biochemical nature of saliva and caries:

Saliva from carious persons	*Saliva from caries-free persons*
1. Low content of tryptophane or similar substance.	1. High content of tryptophane or similar substance.
2. Rapid redox poise drop.	2. Slower redox poise drop.
3. Slow reduction of preformed starch blue.	3. Fast reduction of preformed starch blue.
4. Fast dextrinizing time.	4. Slow dextrinizing time.

2. Saliva from mouths with dental decay is more oxidative; saliva from mouths free from decay is more reductive in nature.

3. These and other studies suggest that dental caries and freedom from dental caries may be associated with the presence of varying amounts of α and β amylase in the saliva.

4. The ingestion of crystalline d-1 tryptophane changes the saliva of individuals with carious teeth so that it has chemical and enzymic characteristics more like the saliva of caries-free persons as shown in the above mentioned tests.

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A NOTE ON THE DURATION OF CARIES INHIBITION PRODUCED BY FLUORIDE APPLICATIONS[1]

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Previous studies (1, 2) have shown that periodic topical applications of sodium fluoride will reduce the incidence of dental caries. These reports indicate that this caries inhibiting effect will continue throughout a 2 year period of treatment. McCauley and Dale (3) have offered the only evidence regarding caries activity after cessation of fluoride treatments. They report an increase in caries in 21 children, aged 2 to 13, within the year following the suspension of a 1 year course of fluoride applications.

The availability of some of the patients on whom a 2 year course of sodium fluoride treatment was completed 3 years ago provided an opportunity to cast more light on the duration of the caries-inhibiting effect of fluoride applications.

Study Group: Of the 100 original patients given fluoride treatments in the Brockton schools between January, 1941, and June, 1943, 39 returned for the fourth and final examination in June, 1946. These patients had received a series of 6 applications with a 0.1% sodium fluoride solution in 1 quadrant of their mouths at intervals of approximately 4 months during the 2 years of the original study. At the time of the fourth examinations the patients were between 16 and 18 years of age and had received no fluoride applications or special attention of any sort for a period of at least 3 years.

Caries Examinations: The amount of caries in each mouth was determined by means of a mirror and probe. Three-quarters of the examinations were made by the original examiner (BGB) and the remainder by the junior author. All examinations were carried out without the examiner knowing which quadrant of the mouth had received the fluoride treatment. Graphic recordings were made of each tooth, showing the extent of the caries present and the size and location of any fillings. Extracted, unerupted or erupting teeth were recorded as such. When areas were found which could not be definitely called either carious or caries-free, they were listed as "questionable change." Under the same heading were listed increases in the size of existing cavities and new fillings placed in areas previously recorded as sound. Changes in the condition of the teeth were worked out for each of the 39 patients showing any new caries or questionable changes on previously intact tooth surfaces.

In the original examination, the amount of caries charted in the test and control quadrants of the 39 patients was about the same, the test quadrants having 87 affected surfaces that were carious and the corresponding quadrants on the other side of the mouth 101.

[1] Received for publication January 5, 1947.

RESULTS

The findings on the examination of the patients who completed the 5 years of study are given in Tables I and II. The increases in caries during the 5-year period are summarized in Table I. The caries increment in the quadrants treated with sodium fluoride is considerably less than that in the untreated or control quadrants. The former showed an increase of 188 definite and questionable cavities as compared with 268 in the latter. The greatest reduction

TABLE I

Caries increase between first and fourth examinations

NO. OF PATIENTS REPORTED FOR BOTH EXAMS.	FLUORIDE TREATED QUADRANTS					UNTREATED CONTROL QUADRANTS				
	First exam.			Re-examination		First exam.			Re-exam.	
	Quadrant	No. of teeth	No. of affected surfaces	New caries	Questionable change	Quadrant	No. of teeth	No. of affected surfaces	New caries	Questionable change
7	L.R.	46	15	23	5	L.L.	49	13	31	3
11	U.R.	71	27	37	13	U.L.	74	30	65	16
9	L.L.	61	16	39	8	L.R.	59	17	53	7
12	U.L.	83	29	42	21	U.R.	82	41	71	22
Totals.... 39		261	87	141	47		264	101	220	48

TABLE II

Caries increase between third and fourth examinations

NO. OF PATIENTS REPORTED FOR BOTH EXAMS.	FLUORIDE TREATED QUADRANTS					UNTREATED CONTROL QUADRANTS				
	Third exam.			Fourth exam.		Third exam.			Fourth exam.	
	Quadrant	No. of teeth	No. of affected surfaces	New caries	Questionable change	Quadrant	No. of teeth	No. of affected surfaces	New caries	Questionable change
7	L.R.	46	15	6	5	L.L.	49	13	12	3
11	U.R.	71	27	21	10	U.L.	74	30	31	10
9	L.L.	61	16	15	8	L.R.	59	17	19	5
12	U.L.	83	29	21	18	U.R.	82	41	31	17
Totals.... 39		261	87	63	41		264	101	93	35

was in the new caries; over the 5-year period there were only 141 definite new cavities in the treated quadrants as compared with 220 in the control quadrants, a reduction of approximately 36%. Table II summarizes the results of the changes during the last 3 years of the study, during which time none of the patients had received any fluoride treatments. Here again the results are definite. The increase in caries in the quadrants originally treated with sodium fluoride is again considerably less than that in the control quadrants. The former showed an increase of 104 definite and questionable cavities as compared with

128 in the latter. Again the greatest reduction was in the new caries; there were only 63 definite new cavities in the treated quadrants as compared with 93 in the control quadrants, approximately a 30% reduction. Table III presents data on the progress of caries in the patient group during the various periods of study.

TABLE III

Caries increase in 39 patients during different periods of study

STUDY PERIOD	TREATMENT	NEW CARIES		QUESTIONABLE CHANGE		TOTAL CHANGES	
		No.	% Reduction	No.	% Reduction	No.	% Reduction
First year of treatment	Test quadrants	14	65	21	0	35	43
	Control quadrants	40		21		61	
Second year of treatment	Test quadrants	37	30	18	36	55	32
	Control quadrants	53		28		81	
Three years without treatment	Test quadrant	63	30	41	−17	104	19
	Control quadrants	93		35		128	
Five year period	Test quadrant	141	36	47	2	188	30
	Control quadrants	220		48		268	

DISCUSSION

Although the number of patients in our study group is small, the group is a larger and more uniform one than that reported upon by McCauley and Dale (3). Therefore, on the basis of our findings it seems justifiable to question their conclusion that suspension of fluoride treatment gives rise to a greater susceptibility to caries. The fact that our method of control, untreated quadrants in the same mouth, is more reliable in small groups of patients than the patient to patient control used by McCauley and Dale adds additional value to our findings as compared with theirs. In short we feel justified in suggesting that notwithstanding the contrary opinion recorded above the effect of fluoride treatments in reducing caries activity persists for a considerable period after such treatments are discontinued.

In most of the patients examined by us the reduction of caries activity on the treated quadrants was apparent on casual examination of the mouth, and in some instances certain treated teeth noted as partially decalcified in the previous examinations had withstood for 4 years further inroads of caries while the corresponding teeth on the opposite sides of the mouth had been largely destroyed.

It is interesting to note that as in previous studies the caries reducing effect is more marked in the upper than in the lower teeth. During the 3 years without treatment the percentage reduction of new decay in the upper teeth was about twice as great as that in the lower teeth.

SUMMARY

A group of 39 patients in which 1 quadrant of teeth had been treated 6 times during a 2-year period with a 0.1% sodium flouride solution were reexamined 3 years after the cessation of such treatments. The treated quadrants still showed approximately 36% less new decay than the untreated quadrants. During the period of suspended treatment the occurrence of new decay was approximately 30% less in the quadrants which had been treated 3 years previously.

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SOLUBILITY OF ENAMEL AS AFFECTED BY FLUORIDES OF VARYING pH[1]

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INTRODUCTION

Research in the field of enamel solubility is of vital importance because it is known that solubility is a contributing factor in dental caries. There seems to be little doubt today that the use of fluorine compounds are a definite aid in reducing enamel solubility, and thus lowering the incidence of dental caries. The literature contains much evidence in support of this belief, both from a clinical standpoint and from experiments performed in vitro.

Miller found that individual teeth appeared to have different rates of decalcification, i.e., tooth solubility, (1) while Pickerell observed that teeth varied in resistance to acids (2). The latter found that the enamel of unerupted teeth was twice as soluble as that of erupted teeth. Dobbs concluded that enamel hardness and resistance to acid decalcification are definitely associated (3).

Karshan and Rosebury (4) have studied the relationship of pH, calcium and phosphorus to the solubility of powdered enamel with lactic, succinic, and malic acids. They stated that the pH change at each time interval is definitely proportional to the dissolved phosphorus at that level.

McClelland observed that the decalcification occurs very rapidly in hydrogen-ion concentrations within the ranges of pH 1.0 to 2.5, because at these intensities of acidity a marked loss of weight was obtained within 30 minutes (5). Many other studies dealing with enamel solubility and the influence of various acids, phosphorus and calcium levels, could be cited.

It has been shown that, within certain limits, where there is an increased fluorine-content in the water supply, there is a greater resistance to dental decay. Evidence submitted from diverse sources shows that when fluorine is added to the diet or the drinking supply of laboratory animals there is a marked reduction in experimental dental decay. The effectiveness of fluorine as a prophylactic measure in preventing dental caries has been demonstrated both in experimental animals (6), and in man (7, 8). Other work (16) has indicated that when there is a greater resistance to tooth decay, there is an increased amount of fluorine in the tooth substance.

Simply knowing that fluorine reduces tooth decay is not necessarily enough, for it is equally as important to find the mechanism by which fluorine acts. Only by a better knowledge of its behavior can we make full use of its poten-

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tialities. As we understand it today, fluorine does reduce the solubility of the enamel, but exactly how the fluorine is incorporated into the tooth structure is still unsolved.

The major chemical component of enamel is hydroxyapatite, $Ca_3(PO_4)_2 \cdot Ca(OH)_2$. It is believed that the soluble fluorides will react with the enamel to form the insoluble fluorapatite, $Ca_3(PO_4)_2/CaF_2$. Compounds of the heavy metals, such as lead fluoride, are supposed to form a compound such as $Ca_3(PO_4)_2 \cdot PbF_2$, which is highly insoluble. Thus, fluorine, by its reaction with the enamel, is presumed to produce a surface which is less soluble than the original. Whether that is accomplished by an adsorption process or by an actual change in the space lattice cannot be definitely stated as yet. We now have various studies underway relating to this problem.

The fact that the enamel is rendered less soluble to the deleterious effects of acids when treated directly with fluorides has been shown by Bibby (9), Volker (10), and Benedict and Kanthak (11), and others.

Volker measured the solubility of enamel and dentin at various concentrations of sodium fluoride (12). His observations seem to prove that fluorine reacts with particles of tooth substances to produce a product that is less soluble. Buonocore and Bibby have pointed out the fact that other fluorides are more effective in reducing enamel solubility than the sodium compounds (13). Lead fluoride was most effective of the compounds studied. Van Huysen and Muhler have found that the fluoride of tin seems to be the best compound to use in this respect (14).

Because of the established beneficial effect of fluorine in reducing enamel solubility and thus inhibiting dental caries, any knowledge of its definite mechanism, or of its use is of vital importance. In order to achieve the maximum inhibiting action of fluorine, we must understand and control all of the various variables involved in its application.

PURPOSE

One of the variables involved in the topical application of fluorine is the pH of the solution. Although it has been shown that fluorides, buffered to an acidity of pH 4.0, reduce the solubility of enamel (13), there is no definite evidence to indicate whether that is the optimum pH for inhibiting solubility or whether possibly some other pH is more effective.

The purpose, therefore, of this experiment is to find the most effective pH of the reagent solution for topical application without damage to tooth tissue and supporting structures. The study was made, using both powdered enamel and whole tooth crowns.

TECHNIQUE AND PROCEDURES

A. *Powdered Tooth Enamel.* One hundred mgm. of enamel from non carious and sound teeth, prepared by the method of Manly and Hodge (15), were placed in a previously tared crucible. These crucibles were of the Royal Berlin type, with finest porosity available. The porosity was estimated by us to be within

the range of 6–8 micra. Five milliliters of a 1 to 500 solution of sodium fluoride were pipetted into the crucible and the contents stirred for 20 minutes. The solution was then filtered under water suction and washed thoroughly with distilled water. After this, a decalcifying solution of .2M, pH 4, acetic acid was added to determine whether or not there had been any protection by the sodium fluoride. Twenty milliliters of acetic acid were placed in the crucible and the contents again stirred for 20 minutes, after which time the solution was filtered, washed and dried in an oven for 3 hours at 38°C. The crucibles were then weighed and if the results varied by more than 0.0001–0.0004 gm. they were discarded. These results are summarized in column 2 of Table I.

As a control, an equal weight of powdered enamel was treated in an acid solution of the same pH but without fluorine. Five milliliters of an acetic acid solution, which was adjusted to the pH used with the fluorine protecting agent,

TABLE I

Showing a comparison and summarization of solubility on treated and untreated powdered enamel at various pH values

("M" number represents difference in weight loss between treated and untreated)

pH	LOSS IN WEIGHT AFTER TREATMENT WITH NaF AND HAC	LOSS IN WEIGHT AFTER TREATMENT WITH HAC	"M" NUMBER
	grams	grams	
2.6	−0.0241	−0.0393	152
3.5	−0.0144	−0.0250	106
4.5	−0.0120	−0.0186	61
5.5	−0.0121	−0.0162	41
6.5	−0.0118	−0.0192	74
7.5	−0.0109	−0.0161	52

were added to 100 mgm. of enamel. The contents were stirred for 20 minutes and washed with distilled water. Then, the decalcifying solution, 20 milliliters of a .2M acetic acid, was added and the contents stirred for 20 minutes. The solution was filtered, washed and dried at 38°C. for 3 hours. Thus, these samples were treated for the same period of time at the same pH as those which were protected with the sodium fluoride and their solubility served as controls. The results are found summarized in Table I, which shows the effects of varying the pH of the solution from 2.6 to 7.5.

In column 4 is a figure which we have arbitrarily called the "M" number. In comparing the fluorine treated samples with the controls, it can be seen that there is a marked difference in solubility. For example, at a pH of 2.6, the enamel treated with sodium fluoride and acetic acid lost 0.0241 gm., but when treated for equal periods of time in acetic acid at the same pH without fluorine, the loss in weight is 0.0393 gm. Therefore, in evaluating the effect of the sodium fluoride, we actually must compute the difference in the loss in weight of the treated with the untreated, or in this case, 0.0241 gm. with 0.0393 gm. Thus, subtracting the two, we get a difference of 0.0152 gm. Instead of expressing this

in actual grams we have merely removed the decimal and called it the "M" number. It is an easy way to express the difference in solubility without referring back to the original weight losses. Naturally the greater the difference in solubility between the treated and the untreated enamel, the higher will be this "M" number, and thus, the greater is the inhibiting action of the fluoride.

B. *Whole Tooth Sections.* Although the most accurate method of studying solubility is by means of powdered enamel, there are certain advantages to be gained by the use of whole tooth sections. With powdered enamel, there are several factors which reduce its similarity to the typical enamel which is exposed in the oral cavity, such as the excessive dehydration during the preparation of powdered enamel, the greater surface area, and the unnatural type of surface that is presented by the powder particles. Consequently, as an adjunct to the work with powdered enamel, we felt that studies made with whole sections cut from freshly extracted teeth would be advantageous in more closely correlating this problem with actual clinical conditions and in offering a further check on our findings already demonstrated on powdered enamel.

Sound and caries-free molar teeth were used and were thoroughly cleaned with scaler and pumice. At no time, from extraction until the end of the experiment, were the teeth allowed to dry. Following cleaning, the crowns were cut off and sectioned into 4 approximately equal parts by means of a diamond wheel under water. The freshly cut surfaces of enamel and dentin were then covered with a thin layer of impression compound so that only the normal outer enamel surface was exposed to the subsequent action of the various solutions. Studies made on the solubility of the compound which was used to cover the freshly cut surfaces showed it to be inert and it exhibited no weight loss when stored in the various solutions for prolonged periods of time. Thus, any weight changes were due entirely to the action of the solution upon the exposed enamel surface.

These sections were then stored in tap water until they reached a constant weight, i.e., did not vary by more than .1 milligram on weighing for 3 consecutive days.

There are several alternative methods for weighing whole sections. After thorough study of these techniques the following procedure was used, since it proved to be not only the most accurate and quickest but also it did not alter the tooth surface by excessive dehydration or treatment with foreign reagents: The section was removed from the water and in order to remove all surface moisture it was dried by means of absorbent tissue and hand air-syringe. It was then immediately weighed. Speed is essential so that the weighing can be finished before dehydration of adsorbed water within the tooth takes place. This adsorbed water is an important factor to be considered since the tooth will continue to lose weight over a long period of time when allowed to dry out in the air.

There is undoubtedly some experimental error to any "wet weight" change method and consequently it can not be considered as accurate as the use of powdered enamel. However, this loss in accuracy is somewhat compensated by the

opportunity to run tests on a relatively normal tooth surface. Since certain factors, such as humidity, porosity of each tooth, and time in weighing, do introduce experimental error, we consider that any weight change of ±.2 milligram on these sections is not of practical significance. Consequently, all the results shown are corrected for this experimental error and actually show changes about .2 milligram. Due to differences in the sizes of sections and thus, variation in surface exposed, all weight changes are expressed not in milligrams but in terms of percent loss from the original weight.

Unfortunately, the inherent variation in individual teeth is extremely great. Their physical and chemical behavior is dependent upon their age, case history, type of tooth, etc. Because of this tremendous variation in teeth, conclusions can not be drawn on tests made on a small number of teeth. A general statement concerning what may be anticipated in the average tooth can be made only after literally hundreds of tests have been made. However, in comparing the action of the fluorine solution with the control of acetic acid at the same pH, it is possible to reduce the number of necessary tests by running the 2

TABLE II

Solubility of whole tooth sections at the various pH values

pH	LOSS IN WEIGHT AFTER TREATMENT WITH NaF AND HAC	LOSS IN WEIGHT AFTER TREATMENT WITH HAC	"M" NUMBER
	%	%	
2.6	−.84	−3.22	2.38
3.5	−.31	−1.71	1.40
4.5	−.52	−.99	.47
5.5	−.74	−.87	.13
6.5	−.50	−.73	.23
7.5	−.20	−.39	.19

solutions on sections of the same tooth. This method permits comparison of the fluoride with the control on sections with approximately the same chemical composition and comparable physical characteristics. In this series of tests we treated 2 sections of each tooth with the fluorine solution and the other 2 sections of the same tooth with the acid control. Therefore, on each series of tests we were comparing the effect of fluorine solutions with acid solutions of similar pH on sections of the same tooth, eliminating to some extent the variables of composition, inherent physical properties, and other factors.

The same pH's were used as with the powdered enamel. However, since the solubility of whole tooth sections is not so great as that for powdered enamel, it was necessary to use longer periods of immersion in order to get an appreciable weight loss. Two sections of the tooth were placed for 60 minutes in an agitated sodium fluoride solution of the designated pH and then were treated, after thorough washing, with the pH 4.0 acetate buffer for 90 minutes. As a control, the other 2 sections of the same tooth were treated for 60 minutes with acetate buffer without fluorine at the designated pH for 60 minutes plus the 90 minutes in the pH 4.0 buffer.

Table II shows the results for the various solutions ranging from pH 2.6 to 7.5. The losses, expressed in percentage, represent the average of at least 3 teeth, or 6 sections.

DISCUSSION

Considering the data for powdered tooth enamel, in column 1, Table I, it can be seen that at a pH of 2.6 there is a marked loss in weight, 0.0241 gm., even when enamel is protected with sodium fluoride. This is due, of course, to the action of the pH 4.0 decalcifying solution. However, when untreated

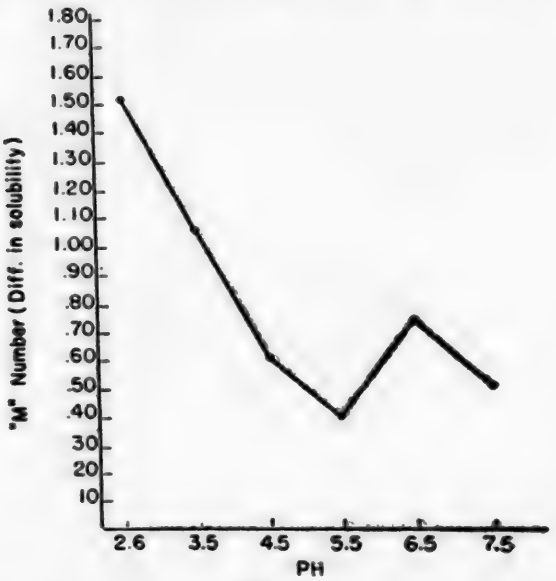

Fig. 1

with sodium fluoride, the enamel showed a much greater solubility, 0.0393 gm., (column 2), when submitted to the same pH for equal periods of time.

At each pH, the solubility of the treated samples is always less than those which are not treated with fluorine. However, as the pH is raised, the difference in solubility between the treated and untreated, the "M" number, becomes less. From the data, it can be seen that the lower the pH of the fluorine solution the more effective it is in reducing enamel solubility.

This trend can be seen graphically in *fig. 1*. The "M" numbers, which express the actual difference in solubility between the treated and the untreated, are plotted against the respective pH's. As the pH is raised the difference in weight loss between the treated and untreated samples becomes less and thus, the "M" number becomes smaller. This curve thus shows the decrease in the protective action of the fluoride as the pH of the solution increases. One exception to this trend can be seen in the fact that the "M" number increases instead

of decreases when going from a pH of 5.5 to 6.5. At this point there is a slight rise in the curve. The reason for this effect will be discussed in a future paper. Although there is an apparent slight increase in the inhibiting action at this pH of 6.5, it is not sufficient to warrant the conclusion that topical solution be buffered to this value. We believe, and it will be shown also with whole tooth sections, that the lower the pH the greater the inhibiting effect will be. From this graph it can be seen that the loss in effectiveness is quite great from a pH of 2.6 down to pH 4.5 and then it levels off.

In looking over these results, one might be inclined to question the use of a solution of 2.6 for topical application, even though it appears to be most effective at this pH, because of the possibility of injury to the tooth structure at this low a pH. We have checked this factor to determine whether there is any appreciable weight loss of enamel when treated with a fluorine solution of this pH. That work is covered in the latter part of the paper and the results secured are sufficient to justify the conclusion that there is no danger of injury to the tooth when treated topically at a pH of 2.6 for limited periods of time.

From the data on the whole sections in Table II, it can be seen that at each pH the percent weight loss is greater for the controls, the untreated sections. For example, at a pH of 2.6 the average loss when treated for 60 minutes with fluorine and a subsequent immersion of 90 minutes at pH 4.0 acetic acid was 0.84%. The average loss, however, for sections of the same teeth in unfluorinated acetic acid solutions at equal pH is 3.22%. The "M" number again represents the difference between the two, or 2.38 in this case. In going down the list, the "M" number decreases as the pH goes up, indicating that the protective action of the fluoride is decreasing. This holds true until a pH of 6.5 is reached, at which level we get a slight rise in the number. This can be seen in *fig. 2*. It should be recalled that this observation was found also when considering the data with powdered enamel.

There is, of course, a difference in the rate of solubility between powdered enamel and whole sections, but these data do follow exactly the results we found previously with powdered enamel, mainly that the lower the pH, the more effective is the fluoride in reducing the solubility of enamel.

The question of the danger in using a fluorine solution of pH 2.6 might arise. In order to check the possibility of injury we placed whole tooth sections in a solution at pH 2.6 for a period of 24 minutes and measured the weight loss. The period of treatment, 24 minutes, was arbitrarily selected since one of the recognized procedures for topical application is to treat each quadrant of the mouth for 4 minutes each with 6 treatments in all. We found the actual weight loss of sections, immersed for 24 minutes in pH of 2.6 sodium fluoride, to be 0.3 mgm. This was also found with sections which were treated for 4 minutes, removed and placed in tap water for a minimum of 3 hours, immersed again for 4 minutes, etc., until they had been intermittently treated for 24 minutes. Again, the average weight loss was 0.3 mgm. We have pointed out previously that we consider the experimental error for the "wet weight" method to be 0.2 mgm. so this weight loss is only slightly above that value.

Using 100 mgm. samples of powdered enamel, we found an average loss of only 0.0030 gm. after 24 minutes of treatment with the pH 2.6 sodium fluoride solution. In view of the solubility effect of various other substances taken into the mouth, and considering that immersion is a more accelerated test than painting or spraying a tooth surface with a fluoride solution, we feel justified in stating that no deleterious effects are produced by the use of fluoride solution of pH 2.6 with the length of treatment commonly used.

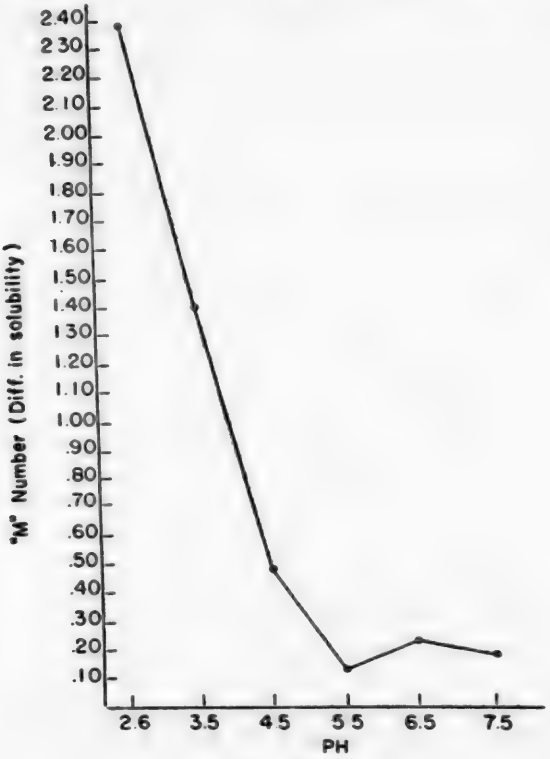

Fig. 2

SUMMARY

By use of both powdered enamel and whole tooth sections, it was found that the lower the pH of the sodium fluoride solution, the more effective is the protecting mechanism in reducing enamel solubility. Tests show a pH of 2.6 not to be injurious to the tooth surface during the time of topical application.

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SOLUBILITY OF ENAMEL PROTECTED BY SODIUM FLUORIDE AND OTHER COMPOUNDS[1]

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INTRODUCTION

Clinical experience, as well as experimentation in vitro, has indicated that the treatment of enamel and dentin with certain compounds will retard the activity of dental caries. For generations dentistry has, on an empirical basis, advocated the use of silver nitrate applied to certain localized areas of the teeth to reduce erosion and decay (1). Rickert (2), for instance, stated that silver nitrate increased the resistance of enamel to decalcification. It was his opinion that this action was due to the clogging of tooth substance with reduced silver ions and that this prevented the penetration of acids. A study of the effectiveness of silver nitrate was made by Hill and Arnold (3). They reported that silver nitrate significantly reduced the solubility of powdered enamel. Other reports on the use of silver nitrate are available but will not be mentioned here.

The effectiveness of fluorine as a prophylactic measure in preventing dental caries has been demonstrated both in man (4, 5), and experimental animals (6, 7). An analysis of a number of carious and non-carious teeth has shown that the teeth with the least amount of caries are those with the greatest amount of fluorine incorporated in their structure (8). Topical application of dilute solutions of sodium fluoride to the tooth surfaces has reduced tooth decay (9) even though analysis of similarly treated tooth surfaces did not show the presence of fluorine (10).

Studies of the chemistry of fluorides, with respect to calcium phosphate, made by MacIntire and Hammond demonstrated the solubility-reducing action of fluorides upon calcium phosphate (11), and Adler, Klein and Lindsay showed the same with hydroxyapatite (12).

Smith and Smith also reported that fluorine actively combines with bone tissue (13). It was Adler, Klein and Lindsay, who predicted that the fluorine might react with the mineral phase of fully formed teeth and give a reduction in solubility like that produced in calcium phosphate (12). Experiments have borne out this prediction, for enamel is rendered less soluble in acids when treated directly with fluorides. Support for this is found in the work of Bibby (9), Volker (15), and Benedict and Kanthak (16).

Volker measured the solubility of enamel and dentin when protected by various concentrations of sodium fluoride (15). His observations seem to

[1] Supported by funds from the Indiana State Health Department and the United States Public Health Service, received for publication February 5, 1947.

establish the fact that fluorine reacts with particles of tooth substance to produce a product that is less soluble. This reaction has been attributed to be similar to that found occurring between fluorine and bone, or other phosphates of calcium. It has been assumed to consist of a change of the apatite to a fluor-apatite. Volker stated that the rapidity with which this reaction takes place makes it seem possible that during the process of drinking water, fluorine from the liquid could be taken up by the teeth, thereby reducing their solubility and caries incidence alike. Dean corroborated this effect by pointing out the fact that the upper anterior teeth are most completely protected by fluorine, for they have the greatest surface contact with the drinking water. There is also a minimum amount of protecting saliva present in this area (17).

Buonocore and Bibby have pointed out that other fluorides are more effective in reducing enamel solubility than the sodium compound (18). According to these authors, lead fluoride was most effective in this process. In repeating the work of Buonocore and Bibby, it was found that the filtration method which they used proved unsuccessful in our hands. It was not possible to use this method because, following the contact between the powdered enamel and the decalcifying solution, particles of the tooth tissues were so reduced that they passed the filter bed. It was, therefore, decided to conduct enamel solubility studies using a modified filtration or volumetric method.

It is the purpose of this experiment to compare (using volumetric method) the protective effect of sodium fluoride on enamel solubility with that of other reagents. The reagents used for comparative purposes are: lead fluoride, uranyl nitrate, zinc fluoride, lead nitrate, copper fluoride, stannous fluoride, silver nitrate, iodoethanoic acid, copper sulphate, sodium iodide, potassium iodate, thorium oxide and secreted human saliva.

PROCEDURE

I. *Solubility of Untreated Powdered Enamel in an Acetate Buffer Solution.* The first step in the procedure was to determine the solubility of 0.1000 gram of powdered enamel in 25 milliliters of acetate buffer solution, adjusted to a pH of 4.0.

A variety of the Royal Berlin crucible, known as the Selas filtering crucible, was used to contain the reagents and powdered enamel. The crucibles had the fine type of filtering bed with an estimated pore size of 7 microns in diameter. These crucibles were layed in a plain porcelain crucible to protect them while being heated over a Meeker burner to establish a constant weight. After the crucibles had been heated, they were stored in a desiccator for at least 6 hours before they were used.

Into each tared crucible there was weighed 0.1000 gram of powdered enamel that was prepared by the method of Manly and Hodge (19).[2] The enamel was

[2] After separating the enamel from the dentin and pulpal tissue, according to the method devised by Manly and Hodge, the enamel was put in a series of standard sieves to obtain fractions of a uniform particle size that remained on the 140 mesh screen and passed the 100 mesh screen. The 140 mesh screen, and contents, were vibrated on a mechanical vibrator

then treated with 25 milliliters of a solution prepared from glacial acetic acid[3], specific gravity $1.049^{\frac{25}{4}}$. This solution was of 0.2 molar concentration and was buffered with a one normal sodium hydroxide solution to a pH of 4.0, measured by a standard potential calomel electrode. The contents of the crucible were stirred for 40 minutes[4] at room temperature. Any debris was removed from the stirring rod by washing it with a few milliliters of distilled water. The crucible was then taken to a water suction filter and the liquid removed. Each crucible was washed carefully and in a uniform manner three times with about five milliliters of distilled water. The washings were removed each time by suction through the filter. It was then taken to an oven and dried at 70° C. for 6 hours. At the end of this period it was placed in a desiccator for 12 hours and weighed. A similar procedure for drying and desiccating was followed until no greater variance than 0.0001 to 0.0002 gram was encountered.

Although this procedure for the measurement of the solubility of unprotected enamel is described separately, actually in each instance two 0.1000 gram portions of enamel were weighed from the same sample. One portion was examined untreated for its solubility; the other was protected with a reagent according to directions which are to follow, and its solubility in acetate buffer compared with that of the unprotected sample. The values thus obtained are listed in Table I.

II. *Solubility of Saliva and Reagent Protected Enamel.* To determine the effect that saliva and reagent solutions had in inhibiting solubility, 0.1000 gram of powdered enamel was weighed into a tared Selas crucible. The enamel portion was then treated with 5 milliliters of the reagent solutions[5]. The

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until no enamel passed. This additional vibrating of the enamel removed all of the smaller particles from the 100 to 140 mesh enamel. The small size of the filter bed pores and the above procedure were sufficient to stop any loss through the filter bed of the crucibles following the action of the decalcifying solution on the enamel.

[3] Apparently lactic acid more closely approaches conditions that exist in the mouth, at least with respect to the acid produced by caries, but the lactic acid is difficult to adjust to a pH satisfactory for this work. Therefore, the work of Volker, Hodge, Wilson and Van Voorhis was followed and acetic acid was used in all of our decalcifications to compare the effect produced by protected and unprotected enamel.

[4] This experiment was started with the assumption obtained from reading the literature that enamel solubility was stopped, or sufficiently decreased, in 18 minutes so that no significant decalcification would be evident after such an interval. With respect to this assumption, some preliminary studies showed that powdered enamel in acetic acid at pH of 4.00 continued to lose a decidedly marked amount of weight (assumed to be decalcification) for at least 90 minutes.

[5] Certain of the reagent solutions used to protect enamel against solubility gave evidence of insolubility in solution of 1/500 concentration. These reagent solutions contained residues. It was obvious that if this undissolved reagent remained on the crucible filter bed, it would be weighed and this weight gain would not contribute to the protection of the enamel. To check this factor, a fine porosity filtering crucible was attached to a laboratory water faucet suction filtering apparatus and all the solutions were filtered. To insure accurate results, and remove fear of contamination, a cleaned crucible was used for each different reagent. The solution that was filtered through the crucible (filtrate) was also used to test the protective action as described under the procedure.

enamel and reagent solution was stirred for 20 minutes and any remaining debris was removed from the stirring rod with a few milliliters (constant volume in each instance) of distilled water. It was then filtered under suction by means of an aspirator, washed with distilled water and filtered as described above. This same procedure was used both with the filtered and unfiltered reagent solutions. To get saliva-protected powdered enamel for this study, about a gram of enamel was mixed in a beaker with 15 milliliters of previously secreted saliva. This mixture was stirred for 60 minutes, after which it was centrifuged, the liquid phase decanted and the powder dried with warm air at 40°C.

TABLE I

A comparison of the weight changes (in grams) which occur when acetic acid at pH 4.00 is combined with powdered enamel unprotected and protected by saliva, filtered and unfiltered reagent solutions

REAGENT	UNFILTERED REAGENT SOLNS.		DIFFERENCES	FILTERED REAGENT SOLNS.		DIFFERENCES
	Unprotected	Protected	A	Unprotected	Protected	B
NaF	−0.0189	−0.0088	0.0101	−0.0275	−0.0128	0.0147
PbF_2*	−0.0189	−0.0077	0.0112	−0.0289	−0.0119	0.0170
$UO_2(NO_3)_2$	−0.0189	−0.0122	0.0067	−0.0272	−0.0138	0.0134
ZnF_2	−0.0258	−0.0093	0.0165	−0.0289	−0.0136	0.0153
$Pb(NO_3)_2$	−0.0189	−0.0047	0.0142	−0.0289	−0.0077	0.0212
CuF_2	−0.0189	−0.0063	0.0126	−0.0272	−0.0119	0.0153
SnF_2	−0.0189	+0.0015	0.0204	−0.0275	−0.0099	0.0176
$AgNO_3$	−0.0258	−0.0180	0.0078	−0.0272	−0.0173	0.0099
CH_2ICOOH	−0.0258	−0.0170	0.0088	−0.0289	−0.0240	0.0049
$CuSO_4$	−0.0258	−0.0140	0.0118	−0.0270	−0.0240	0.0188
NaI	−0.0275	−0.0221	0.0054	−0.0270	−0.0179	0.0191
KIO_3	−0.0275	−0.0238	0.0037	−0.0270	−0.0188	0.0182
ThO_2	−0.0275	−0.0074	0.0201	−0.0275	−0.0245	0.0030
Saliva	−0.0248	−0.0219	0.0029			

* Saturated solution approximately 1/600 concentration.

Twenty milliliters of the decalcifying solutions were then added to the dried treated enamel and the combination was stirred for 20 additional minutes. It was then filtered, washed, dried, desiccated and weighed in the same manner as described above.

Again it should be pointed out that although the procedure for measuring the solubility of unprotected and saliva - and reagent-protected enamel are described separately the solubility of unprotected, saliva - and reagent-protected enamel were actually carried out on portions of the same samples of the enamel and acetic acid in each of the respective instances. At least 12 measurements were made not differing from each other by more than 0.0002–0.0004 gram. The data in Table I are an average of the values for the measurements.

DATA

Table I shows the data comparing the effect of saliva and the various filtered and unfiltered reagent solutions that are believed to have some protecting action

with respect to enamel solubility. The first column of the table is a list of the reagent solutions used to protect the enamel. The second column of the table contains values expressing the weight changes in grams when unprotected enamel was treated with the acetate buffer. The third column of the table contains weight change data in grams obtained when enamel protected by unfiltered reagents was treated with the acetate buffer. The fourth column lists the values obtained by comparing the weight changes of protected and unprotected enamel where unfiltered reagent solutions were used for this enamel protection. The fifth column again shows weight changes in other samples of unprotected enamel treated with the acetate buffer. The sixth column contains data which show the weight change in grams obtained when similar samples of enamel protected by filtered reagent solutions were treated with the acetate buffer. The seventh column of the table lists the values obtained by comparing the weight changes of protected and unprotected enamel where filtered reagent solutions were used for this enamel protection.

It will be noted in column 3, line 7 of the table, along with the data on stannous fluoride, that the enamel protected with unfiltered stannous fluoride gave a value of +0.0015 gram. This value in line 7 means that the powdered enamel, although protected with unfiltered stannous fluoride, even following combination with the acetic acid, weighed 0.0015 gram more than did the original 0.1000 gram of powdered enamel used to make the test. This is the only reagent in this study which showed this over-all weight gain. When filtered stannous fluoride was used there was, however, a loss and not an over-all gain in weight.

This table also shows that non-fluorine containing reagents, such as lead and uranyl nitrate, copper sulphate, thorium oxide and silver nitrate, are usable reagents for the inhibition of enamel solubility. These data add emphasis to the idea that much is yet to be learned about enamel solubility itself, and that this problem in basic chemistry should be more thoroughly studied (20). There is a possibility, for instance, that a gravimetric or volumetric analytical technique might yield information of value in this field.

It is of interest to observe, in the last line of the table, that saliva residues protect enamel against solubility.

The data show that different samples of unprotected enamel showed differences in solubility. These different values for unprotected enamel solubility were not only due to the difference in enamel samples but also due to the fact that different samples of acetate buffer were used. This variation in samples of enamel and acetate buffer were of no real significance because the same samples of both were used in the comparison between the solubility of each of the respective reagent protected and unprotected enamel.

DISCUSSION

The evidence presented in this paper is the result of a study of the enamel solubility reducing effect of saliva and various chemical reagents. It is evident, from Table I, that a solution of sodium fluoride is a satisfactory agent to be used to reduce enamel solubility. The table, however, shows that other agents are

just as good or even better. A solution of stannous fluoride in this experiment has proved the most worthwhile agent to reduce solubility of powdered enamel.

It is of interest to note that Table I shows that in most instances filtered reagent solutions protect the enamel against solubility better than do unfiltered reagent solutions. In some instance, there was a marked difference in favor of the filtered solutions. When zinc fluoride, tin fluoride, iodoethanoic acid and thorium oxide solutions were used, the opposite effect was evident. No explanation is offered for this phenomenon.

Bibby (9) and Armstrong and Knutson (21) and others have reported the results of their experience in the inhibition of caries by topically applied sodium fluoride. Apparently, they have been able to reduce tooth decay for at least 3 years following one course of treatments. This would indicate that the fluorine is not only very quickly taken up by the tooth surfaces, but that once it is taken up it is held very tenaciously by the enamel.

In the present study it was shown that enamel solubility was reduced somewhat by uranyl nitrate. Since this material is radioactive and this radioactivity may be estimated by means of the Geiger-Mueller counter, treated and untreated powdered enamel samples were sent to the Indiana University Physics Department where Professor Charles Allan Michell measured them for us. A few tests showed that once the enamel picked up uranyl nitrate 1 hour of washing with distilled water failed to remove the attached radioactive material. Saliva treated enamel also picked up an appreciable amount of uranyl nitrate from solution. The treatment of the powdered enamel was as follows: Into a Selas crucible 0.1000 gram of powdered enamel was weighed and stirred with 5 milliliters of 1/500 solution of uranyl nitrate for 20 minutes. This was dried at 70°C. A second sample of powdered enamel was treated with the similar sample of uranyl nitrate solution for a similar period of 20 minutes, after which it was washed for 1 hour with distilled water and dried. A third sample of 0.1000 gram of enamel was washed with previously secreted saliva, dried, and then treated with the 1/500 uranyl nitrate solution. A fourth, or control, sample of 0.1000 gram of powdered enamel was untreated. These treated and dried samples of enamel were placed between sheets of scotch tape and placed around a counter-tube shielded from the outside by a copper ring. Samples of uranium oxide powder were also placed upon scotch tape and counted. Table II shows the data obtained.

The uranium oxide standards counted showed that individual samples varied from 1 to 2%, but a number of samples varied 10%. These measurements were equivalent to 149 $\pm$ 10% counts per minute, per milligram, or, an equivalent of about 169 counts per minute, per milligram of uranium. When the enamel was treated with a 1/500 solution of $UO_2(NO_3)_2$ dried and 100 milligram samples placed on the counting chamber between layers of scotch tape there were 119.9 $\pm$ 2.6 counts, or equivalent of 0.71 milligrams of uranium. When the 1 hour washed sample of enamel protected by 1/500 solution of uranyl nitrate was attached to the counter, the count was 144 $\pm$ 1.6, or an equivalent of 0.853 milligrams of uranium. Since the equivalent values of uranium were determined

by dividing the counts by 169 counts per minute, per milligram of uranium, and this value is probably in error to about ±10%, the equivalent values of uranium are not too accurate.

This study, it is believed, however, does definitely demonstrate that once tooth enamel does pick up material it is held very tenaciously even though washed with distilled water at a pH of 5.5–6.0. It also shows that saliva residues upon the enamel particle surfaces do permit an appreciable amount of uranium to become added to its surface. More work using other radioactive reagents, preferably one containing fluoride, should be undertaken both to establish greater accuracy and to determine the variety and type of substances which may thus attach itself to powdered enamel.

From a calculation of the molarity, or percentage of fluorine in a compound, there appears to be no relationship between the amount of fluorine contained

TABLE II

Tenacity with which uranium from uranyl nitrate solution $UO_2(NO_3)_2$ adhered to powdered enamel in spite of washing with water

(pH 5.5 to 6.0)

MATERIAL	COUNTS MINUTE	COUNTS/MIN./MG.	COUNTS/MIN./MG. OF URANIUM	EQUIVALENT TO MG. URANIUM
Background counter blank	29			0
Background scotch tape and ring	29			0
Untreated enamel (318.5 mgm. in scotch tape)	28			0
Uranium oxide		149 ± 14.9	169	
Uranyl nitrate and enamel dry		119.9 ± 2.6		0.71
Uranyl nitrate enamel washed (1 hr.)		144 ± 1.6		0.853
Enamel salivated and uranyl nitrate		98 ± 3.0		0.58

in any of the reagents and their ability to reduce enamel solubility. Sodium fluoride, for instance, contains the greatest amount of fluorine incorporated in the molecule, but stannous fluoride, with approximately half of the amount of incorporated fluorine, is more than twice as effective in its ability to reduce enamel solubility. It must be concluded, therefore, that there are other factors to be considered beside the fluorine content of these compounds. Some of the following might be considered: (1) The dieletric constant of the molecules; (2) The calcium salt that is formed and its dissociation constant; (3) The manner in which the cation enters into equilibrium with the apatite molecules; and, (4) The ionization K of the salt formed by the reaction outlined in (2) and (3) above.

This work, and that of others, shows that fluorides and other compounds do protect enamel against solubility. Volker, Hodge, Wilson and Van Voorhis (22) showed, using the radioactive isotope of fluorine F^{18}, that small amounts of fluorine, according to the Freundlich adsorption isotherm, were adsorbed by

dentin, enamel, bone and hydroxyapatite. Armstrong, however, was unable to find fluorine upon the enamel surfaces of teeth treated topically with sodium fluoride. Gruner (23) was unable, using X-ray diffraction methods, to show the presence of adsorbed, combined or otherwise added fluorine, or other ions in the samples of reagent treated enamel which were sent to him from this laboratory. These samples had been treated for 40 minutes with the dilute fluorides and other compounds. Since Gerould (24) and others have shown that with higher concentrations of reagent solutions, measureable and even visible changes take place, it is assumed that the Roentgen ray diffraction pattern method is not sensitive enough to reveal changes obtained with the dilute reagent solutions used in this study.

There is, therefore, still a unique problem facing those investigators who are interested in the manner in which fluorides protect enamel against solubility in weak acids. In the first place, enamel solubility has not been satisfactorily explained, and secondly, though it is known that certain substances do interfere with this solubility, the mechanism especially when dilute solutions are used will have to be more completely studied.

When unfiltered stannous fluoride is used to protect enamel solubility, the weight of the supposedly decalcified enamel increases. This may be due to the fact that undissolved reagent remains on the crucible filter bed, or that a new substance which is insoluble is formed by the reagent enamel. Preliminary tests in this laboratory have led us to believe that the latter phenomenon is operating here, and that it also probably takes place to a lesser degree with some of the other reagents.

SUMMARY

1. More evidence is offered to substantiate the fact that substances other than sodium fluoride may be used in reducing enamel solubility.

2. Stannous fluoride is an effective compound to use for reducing enamel solubility.

3. Filtering the reagent solution does not seem to impede the action of this and certain of the other solutions if they have a sufficiently low solubility.

4. Very indirect and imperfect evidence is given to show that reagents taken up by powdered enamel are tenaciously retained following washing with water.

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STUDIES IN DENTAL PUBLIC HEALTH ADMINISTRATION[1]

II. The Rôle of Roentgenograms in Public Health Dental Surveys

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It is now generally accepted that X-ray examination is an essential part of diagnosis and treatment in children's dentistry (1, 2, 3). In addition to its value in detection of carious lesions of the teeth, the roentgenogram is necessary to determine the progress of growth and development of the child's dental apparatus (4).

While the importance of X-ray examination in the treatment of the individual patient is realized, the question of its importance in public health survey work has often been raised. For the most part, public health dentistry deals with repair of carious lesions and the prevention of premature loss of children's teeth by preventing the carious process from spreading to the pulp. The X-ray would therefore be used primarily to aid in the detection of carious lesions in dental survey work. Sognnaes (5) found an increase of 4.7% in the detection of carious surfaces using X-ray examination. His series consisted of only 32 children, and his clinical examination lasted 1½ hours per patient, which is not feasible for public health dental surveys.

The difficulties involved in performing mass dental X-ray studies are great, although they can be overcome (6). The cost of roentgenographic equipment and the lack of facilities for their proper use, plus the paucity of funds available for dental public health work, necessitate careful evaluation of the role of the roentgenogram in dental public health surveys. It was with this in mind,—to find whether the addition of X-ray examination produced a great enough increase in the detection of caries to warrant its routine inclusion in surveys,—that this study was undertaken.

METHOD

In January 1945 a survey was performed in the schools of the Borough of North Arlington on 2540 children varying in age from 5 to 18 years. This survey was performed in the school classrooms, using ordinary school lighting and mouth mirrors and explorers, by a dentist selected by the local dental society (7), with the aid of a teacher or a school nurse to record the observations. The number of decayed, missing and filled deciduous and permanent teeth, as well as the teeth requiring extraction, was determined for each child, and then tabulated by age.

[1] Received for publication January 9, 1947.

In order to select the "experimental" group, approximately every third child in each class, chosen purely at random, was selected for further examination. The number of children so chosen was 729. In September, 1945, these children were examined in the North Arlington dental clinic, using standard dental equipment and adequate lighting, with mouth mirrors and explorers, and, in addition, bite-wing radiographs. In order to reduce the possibility of variation in the interpretation of just what constitutes a carious lesion, the examinations with mouth mirrors, explorers and X-rays were performed by one dentist (L. E. G.). Although several months had elapsed between the original survey and the examination of the sample group, it was felt that the random selection of the children would tend to obviate the differences caused by the appearance of new carious lesions in the interim. The results of the examination of the sample group were recorded in the same manner as with the whole group, but observations were made from examination with mouth mirror and explorer, and mouth mirror, explorer and X-ray, the difference between the 2 examinations being the additional lesions disclosed by X-ray examination.

RESULTS

Comparing the Number of Decayed Teeth as Determined With and Without X-Rays in the Sample Survey

The differences seen with the X-ray examination are essentially in the number of decayed teeth which require fillings, since missing teeth, and teeth which require extraction can be ascertained, in the age groups we are dealing with, by mouth mirror and explorer examination alone. A tabulation of the data on decayed teeth requiring fillings, as determined with and without the X-ray, was therefore made (Table I). The distribution of the children in each age group according to the number of carious teeth in no way resembled a normal curve. Similarly the distribution of the 729 children at all ages according to the number of carious teeth, did not resemble a normal curve, as may be seen from Table II and *fig. 1*. There is a concentration of children at the low numbers of carious teeth. In distributions of this type, the arithmetical mean or average is a poor measure of the characteristics of the distribution. For this reason, the median (Q_2) was chosen to compare the distributions of the number of decayed teeth requiring fillings seen with mouth mirror and explorer with and without X-ray examination. The median is the number of decayed teeth below which and above which half of the 729 children occur. These can readily be determined from Table II as:

Q_2 with X-rays = 2.75 decayed teeth; Q_2 without X-rays = 1.70 decayed teeth.

It is known that the addition of an X-ray examination to the routine examination with mouth mirror and explorer will disclose more carious lesions, or occasionally no additional lesions, but usually it will not decrease the number of diagnosed carious lesions. The difference in the number of decayed teeth requiring fillings, as determined by examination with mouth mirror, explorer and X-ray, and mouth mirror and explorer without X-ray cannot therefore be

due to chance, since the differences necessarily occur in the same direction. Thus the medians given above for the "d" and "D" distributions do evidently not differ by chance. It might nevertheless be of some value to answer the question as to whether a difference of the indicated magnitude, i.e., 2.75–1.70, could occur by chance when utilizing only 729 children. Performing the significance test on medians in the usual manner, we find that the difference of 1.05 decayed teeth is 4.8 times its standard deviation and would thus be highly significant.[2]

TABLE I

Summary of data on decayed teeth in sample survey of 729 children

AGE AT LAST BIRTHDAY	NUMBER OF CHILDREN	DECAYED DECIDUOUS TEETH (d)		DECAYED PERMANENT TEETH (D)		TOTAL DECAYED TEETH (d & D)			TOTAL CARIOUS TEETH PER CHILD (d + D/NO. CHILDREN)		
		with X-Ray	no X-Ray	with X-Ray	no X-Ray	with X-Ray	no X-Ray	Diff.	with X-Ray	no X-Ray	Diff.
5	12	53	30	1	1	54	31	23	4.50	2.58	1.92
6	58	184	139	22	22	206	161	45	3.55	2.78	0.77
7	61	195	165	75	75	270	240	30	4.43	3.93	0.50
8	63	168	138	92	87	260	225	35	4.13	3.57	0.56
9	58	131	116	83	76	214	192	22	3.69	3.31	0.38
10	58	75	63	91	76	116	139	27	2.86	2.40	0.46
11	58	62	57	114	107	176	164	12	3.03	2.83	0.20
12	54	6	6	139	98	145	104	41	2.69	1.93	0.76
13	59	3	3	216	176	219	179	40	3.71	3.03	0.68
14	69	3	3	255	173	258	176	82	3.74	2.55	1.19
15	71	1	1	359	270	360	271	89	5.07	3.82	1.25
16	61	0	0	218	149	218	149	69	3.58	2.44	1.14
17	37	0	0	187	141	187	141	46	5.05	3.81	1.24
18	10	0	0	29	18	29	18	11	2.90	1.80	1.10
Total.....	729	881	721	1881	1469	2762	2190	572	3.79	3.00	0.79

The median number of decayed teeth was also computed for the children in each age group. The value using X-rays was always higher than that without the X-rays. While the differences are not due to chance for the reasons mentioned above, the magnitudes of the differences could, in other circumstances,

[2] The standard deviation of a median may be calculated from the general formula

$$\sigma_{Q_2} = 50/y\sqrt{N}$$

where y is the ordinate of the frequency distribution, expressed in percentage per unit of abscissa, corresponding to the median. To determine y the distributions in Figure I were first smoothed by free-hand curves. The ordinates at the medians with and without X-rays were then read as 82 and 90 children respectively, or, dividing by 729, as 11.4% and 12.5%. According to the above formula σ_{Q_2} is then computed as 0.16 and 0.15 for the two groups respectively. The standard deviation of the difference in the two medians is then given by:

$$\sigma_{\text{difference}} = \sqrt{(0.16)^2 + (0.15)^2} = 0.22$$

have been explained by chance in some of the age groups, because of the limited numbers of children involved in each age group.

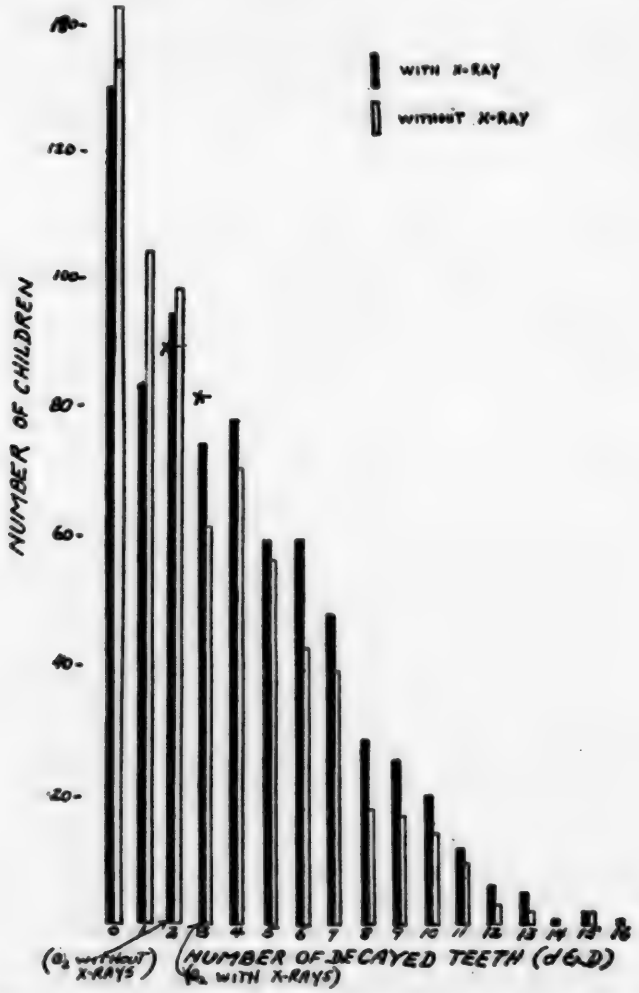

Fig. 1. Distribution of 729 Children at All Ages in Sample Survey, by Number of Carious Teeth.

Comparing the Number of Decayed, Missing, and Filled Teeth, as Determined With and Without X-Rays in the Sample Survey

Since it is generally accepted procedure in public health circles to discuss dental caries experience in terms of decayed, missing and filled teeth (def[3] and

[3] The "e" in the expression def refers only to deciduous teeth requiring extraction, since it is impossible to determine the caries experience of missing deciduous teeth (8).

TABLE II

729 children at all ages in sample survey, by distribution of number of carious teeth

NUMBER OF CARIOUS TEETH	NUMBER OF CHILDREN	
	with X-Ray	no X-Ray
0	130	191
1	84	105
2	95	99
3	75	62
4	79	71
5	60	57
6	60	43
7	48	39
8	29	17
9	25	16
10	19	13
11	11	9
12	6	3
13	4	2
14	1	0
15	2	2
16	1	0
Total.....	729	729

TABLE III

Sumary of dental caries experience in sample survey of 729 children

AGE AT LAST BIRTHDAY	NUMBER OF CHILDREN	DEF TEETH		DMF TEETH		TOTAL def & DMF TEETH			def & DMF TEETH PER CHILD		
		with X-Ray	no X-Ray	with X-Ray	no X-Ray	with X-Ray	no X-Ray	Diff.	with X-Ray	no X-Ray	Diff.
5	12	62	39	1	1	63	40	23	5.25	3.33	1.92
6	58	263	218	36	36	299	254	45	5.16	4.38	0.78
7	61	307	277	92	92	399	369	30	6.54	6.05	0.39
8	63	302	272	157	152	459	424	35	7.29	6.73	0.56
9	58	200	186	166	159	366	345	21	6.31	5.95	0.36
10	58	138	126	246	231	384	357	27	6.62	6.16	0.46
11	58	95	90	292	285	387	375	12	6.67	6.47	0.20
12	54	15	15	399	358	414	373	41	7.67	6.91	0.76
13	59	6	6	523	483	529	489	40	8.97	8.29	0.68
14	69	4	4	738	656	742	660	82	10.71	9.52	1.19
15	71	1	1	900	807	901	808	93	12.69	11.38	1.31
16	61	0	0	776	707	776	707	69	12.72	11.59	1.13
17	37	0	0	524	478	524	478	46	14.16	12.92	1.24
18	10	0	0	129	118	129	118	11	12.90	11.80	1.10
Total.....	729	1393	1234	4979	4563	6372	5797	575	8.74	7.95	0.79

DMF teeth), these data were determined and tabulated (Table III). The distribution of the 729 children at all ages according to the number of def and DMF teeth (Table IV) resembles a normal distribution more closely than did

the distribution of carious teeth (d and D teeth), although it is still positively skewed (*fig. 2*). The mean (m), however, will more adequately describe this distribution than will the median, and therefore a comparison of the means of the frequency distributions of the def and DMF teeth obtained with and without

TABLE IV

729 children at all ages in sample survey, by distribution number of def and DMF teeth

NUMBER OF def & DMF TEETH	NUMBER OF CHILDREN	
	with X-Ray	no X-Ray
0	19	26
1	13	22
2	28	35
3	28	18
4	54	61
5	50	65
6	61	64
7	72	74
8	70	76
9	60	59
10	59	48
11	40	43
12	30	31
13	29	23
14	21	13
15	15	17
16	20	12
17	13	14
18	12	10
19	7	5
20	7	5
21	7	3
22	8	3
23	4	2
24	0	0
25	1	0
26	0	0
27	1	0
Total.....	729	729

X-ray examination, was made. These means can readily be determined from Table IV as:

m with X-ray = 8.74 def and DMF teeth per child; m without X-ray = 7.95 def and DMF teeth per child

The difference of 0.79 in the 2 means cannot be due to chance for the reason already mentioned,—that the addition of an X-ray examination to the routine examination with mirror and explorer will disclose more carious lesions, and hence more def and DMF teeth. If the significance of the difference were

nevertheless tested by the usual method, we would find a statistically significant difference, since the difference is 3.2 times its standard deviation.[4]

The mean number of def and DMF teeth was also computed for the children in each age group. The value using X-rays was, of course, always higher than that without the X-rays. While the differences are certainly not due to chance,

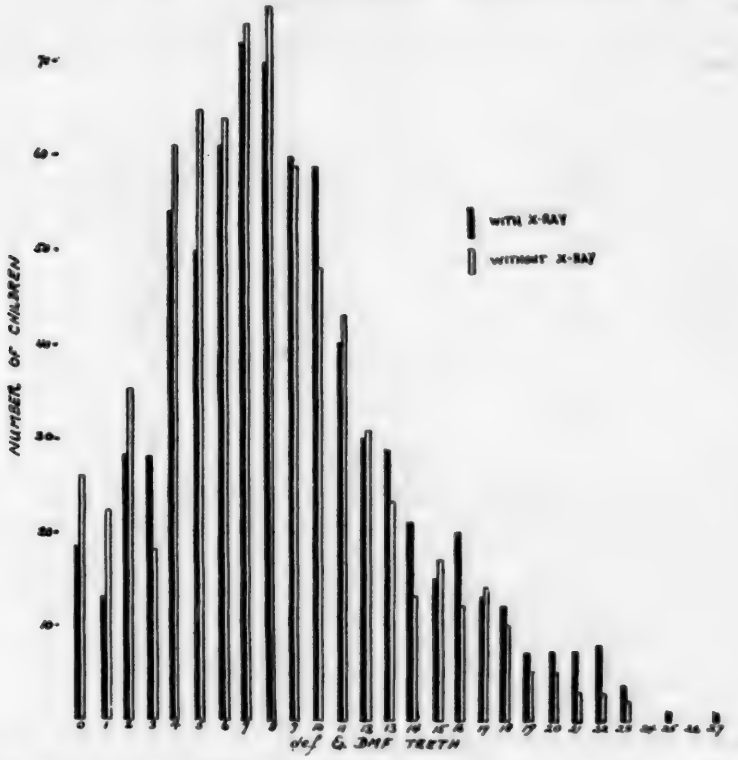

Fig. 2. Distribution of 729 Children at All Ages in Sample Survey, by Number of def and DMF Teeth.

some of the differences could have been explained by chance in the usual situation.

[4] The standard deviation of a mean may be calculated from the formula:

$$\sigma_m = \sigma/\sqrt{N}$$

Where σ is the standard deviation of the distribution in Table IV, computed as:

$$\sigma = \sqrt{\Sigma(x - m)^2/N}, \text{ or more conveniently, as } \sigma = \sqrt{[N\Sigma x^2 - (\Sigma x)^2]/N^2}$$

σ is computed as 4.95 and 4.56 def and DMF teeth for the distribution with and without X-rays respectively. Accordingly, σ_m is 0.18 and 0.17 for the two groups respectively. The standard deviation of the difference in means is then given by:

$$\sigma_{\text{difference}} = \sqrt{(0.18)^2 + (0.17)^2} = 0.25$$

Comparing the Sample Survey with the Master Survey

The results of the master survey are presented in Table V, while in Table VI is presented a comparison of the results of the sample survey with those of the master survey. The mean number of def and DMF teeth per child in the sample survey with X-ray examination are in all cases larger than the corresponding means of the master survey. These differences cannot be ascribed to chance regardless of the outcome of the usual significance tests for reasons already mentioned. The usual significance tests would indicate that all the differences

TABLE V

Summary of dental caries experience in master survey of 2540 children

AGE GROUP	NUMBER OF CHILDREN	def TEETH	DMF TEETH	TOTAL def & DMF TEETH	def & DMF TEETH PER CHILD
5–6	200	861	145	1006	5.03
7–9	619	1847	1616	3463	5.59
10–12	773	847	3759	4606	5.96
13–15	618	85	6216	6301	10.195
16–18	330	5	3759	3764	11.41
Total.....	2540	3645	15495	19140	7.54

TABLE VI

Table of comparison of sample survey of 729 Children and master survey of 2540 children

AGE GROUP	def PER CHILD			DMF PER CHILD			TOTAL def & DMF PER CHILD		
	Sample Survey		Master survey	Sample Survey		Master survey	Sample Survey		Master survey
	with X-Ray	no X-Ray		with X-Ray	no X-Ray		with X-Ray	no X-Ray	
5–6	4.64	3.67	4.31	0.53	0.53	0.73	5.17	4.20	5.03
7–9	4.45	4.04	2.98	2.28	2.21	2.26	6.73	6.25	5.59
10–12	1.46	1.36	1.10	5.51	5.14	4.86	6.97	6.50	5.96
13–15	0.06	0.06	0.14	10.86	9.78	10.06	10.91	9.83	10.195
16–18	—	—	—	13.23	12.06	11.39	13.23	12.06	11.41
Average.....	1.91	1.69	1.44	6.83	6.26	6.10	8.74	7.95	7.54

are significant, except for the 2 age groups, 5–6 and 13–15. Similarly, at all ages the difference between the 2 means of 8.74 obtained in the sample survey with X-rays and 7.54 in the master survey without X-rays is certainly not due to chance. If, nevertheless, the customary significance test is made, in spite of the non-chance character of the difference, we note that the difference is almost 6 times its standard deviation.

When the mean number of def and DMF teeth per child of each age group of the sample survey without X-ray examination are compared with the means of the corresponding age groups of the master survey, we find no case where there is clear-cut significance (probability of the difference being due to chance

between 1 and 5 per cent), the differences are in different directions, i.e., sometimes the means of the sample are greater than the means of the master survey, and sometimes the reverse holds true. Comparison of the overall means is not clearly significant either. The mean for the sample survey without X-rays is 7.95 and that for the master survey is 7.54 def and DMF teeth per child. The difference of 0.41 is only 2.1 times its standard deviation and this is not clearly significant, since it could be exceeded about 3% of the time by chance.[5]

It should be noted that the qualifications with respect to the operation of chance which were made previously when applying the usual significant test, do not need to be made here. Since neither the master survey nor the sample survey in this comparison used X-ray examinations, there is accordingly no reason why one group must necessarily give higher values than the other. We are merely testing whether examinations performed in schools are significantly different from examinations performed in dental clinics. Since the results show no clear-cut significance, and since the differences vary in sign, it appears that chance alone may be responsible for the differences in the average def and DMF teeth per child as ascertained by examinations in schools and in the clinic without benefit of roentgenographic examination.

DISCUSSION

Three principal comparisons have been performed: 1) median decayed teeth requiring fillings in the sample survey with X-ray examination versus median decayed teeth requiring fillings in the sample survey without X-ray examination, and mean def and DMF teeth per child in the sample survey with X-ray examination versus mean def and DMF teeth per child in the sample survey without X-ray examination; 2) mean def and DMF teeth per child in the sample survey with X-ray examination versus mean def and DMF teeth per child in the master survey without X-ray examination; and 3) mean def and DMF teeth per child in the sample survey without X-ray examination versus mean def and DMF teeth per child in the master survey (without X-ray examination).

In the first 2 analyses, it was shown that if a large enough sample is taken the differences are statistically significant in the usual sense. It may be argued, of course, that the difference must always be significant, regardless of its magnitude and the number of children, because of the real additive nature of X-ray examinations. Whether the difference be called statistically significant or not, the practical significance of the difference must be taken into account here. In public health survey work we are primarily concerned with the need of com-

[5] The standard deviation appropriate to the mean of 7.95 was given in the previous footnote as 0.17. The standard deviation for the mean of 7.54 for the master survey was computed by assuming that the σ of the distribution of the 2540 children with respect to def and DMF teeth per child could be taken as equal to 4.56, the σ obtained in the sample survey. Then $\sigma_{\bar{m}} = 4.56/\sqrt{2540} = 0.09$.

Consequently, $\sigma_{difference} = \sqrt{(0.17)^2 + (0.09)^2} = 0.19$.

Although the 729 children included in the sample survey were a part of the 2540 of the master survey, almost a year had elapsed between the two surveys and for this reason the two groups were treated as independent of each other.

munities for dental care. For practical purposes, a difference of 1 or 2 carious teeth per child does not change the need for dental care when there is always a basic average, determined by examinations with mouth mirror and explorer alone, which calls for dental care. This is further borne out when we examine the dental needs, as measured by deDMr (the teeth requiring fillings or extractions), appearing in Table VII. From our data we see that the dental needs of the community under observation are not appreciably different as observed with and without roentgenographic examination. The fact that the deDMr per child ascertained from the master survey equals or exceeds the values obtained from the sample survey with X-ray examination in the older age groups, may perhaps be attributed to the higher average of filled teeth (F) per child in the sample group over the master group. This may be due to a peculiarity of our sampling technique or to the possibility that the children selected for the sample group were motivated to have their dental needs taken care of during

TABLE VII

AGE GROUP	deDMr PER CHILD			F PER CHILD	
	Sample Survey		Master Survey	Sample Survey	Master Survey
	with X-ray	no X-ray			
5–6	4.00	3.03	3.43	—	—
7–9	4.49	4.01	3.92	0.88	0.99
10–12	2.29	2.52	2.64	3.00	2.51
13–15	4.38	3.32	4.38	5.60	4.61
16–18	4.24	3.07	4.89	7.35	4.61
Average.....	4.03	3.24	3.73		

the interim before the second examinations took place, so as to make a better impression upon their teachers.

The differences between the findings with and without X-ray examination, which we choose to believe are not significant from a practical point of view, are depicted graphically in *figs. 3, 4* and *5*. The differences in the d and D and the def and DMF teeth per child appear to be smallest about age 11, and increase as the children become older. This was to be expected, when we consider that X-ray examination will essentially disclose interproximal caries which cannot readily be seen when all the teeth are in position. When the permanent dentition is in the process of erupting, the interproximal surfaces of the teeth are much more visible than when all the teeth are in contiguity, as occurs in older age groups. This corresponds with the clinical observation that interproximal caries appears to increase after the permanent dentition is fully erupted.

Since, from our data, there appears to be no consistent statistical difference between examinations conducted in schools and in clinics without roentgenographic examination, we must conclude that careful examinations performed in survey work in schools will give a practical index of the dental needs of the school population.

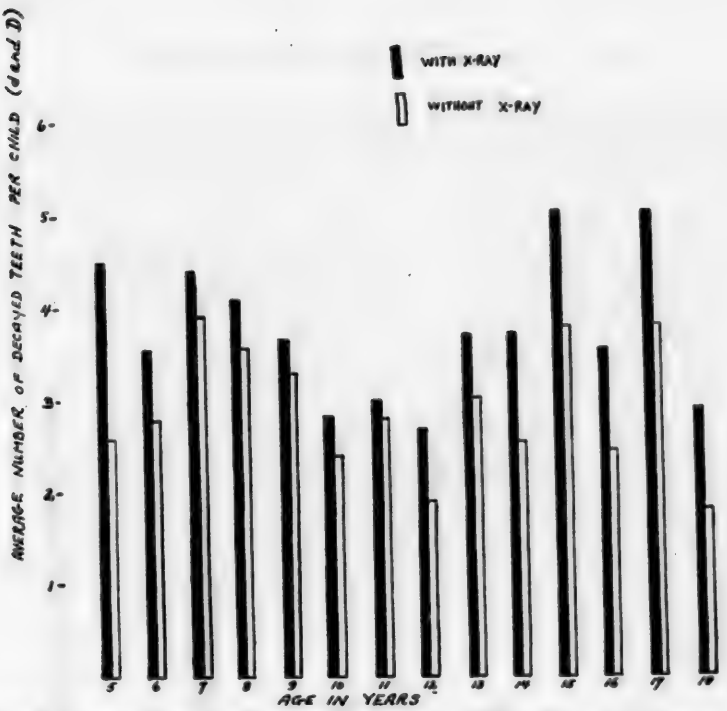

Fig. 3. Average Number of Decayed Teeth (d and D) Per Child at Different Ages Among 729 Children of Sample Survey.

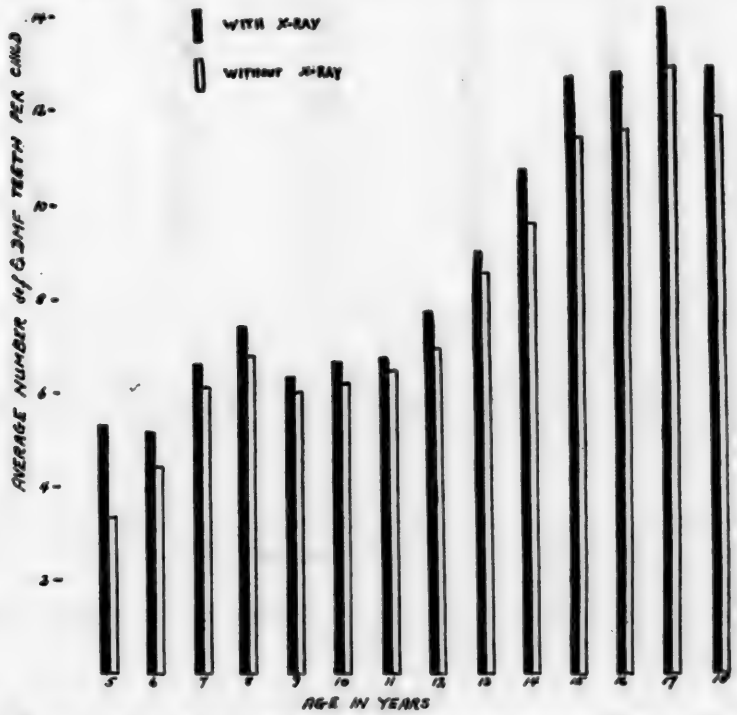

Fig. 4. Dental Caries Experience for Different Ages, by Average Number of def and DMF Teeth per Child of Sample Survey of 729 Children.

Although our data lead us to the conclusion that X-rays afford no practical advantages in dental public health surveys, considering all the difficulties concomitant with their use in school examinations, the same conclusion does not hold for examinations performed for research purposes. When studies are undertaken to determine the efficacy of measures taken to prevent or retard caries, the factor of chance comes into play, and the statistical significance of oftentimes small differences in the average number of carious teeth per child,

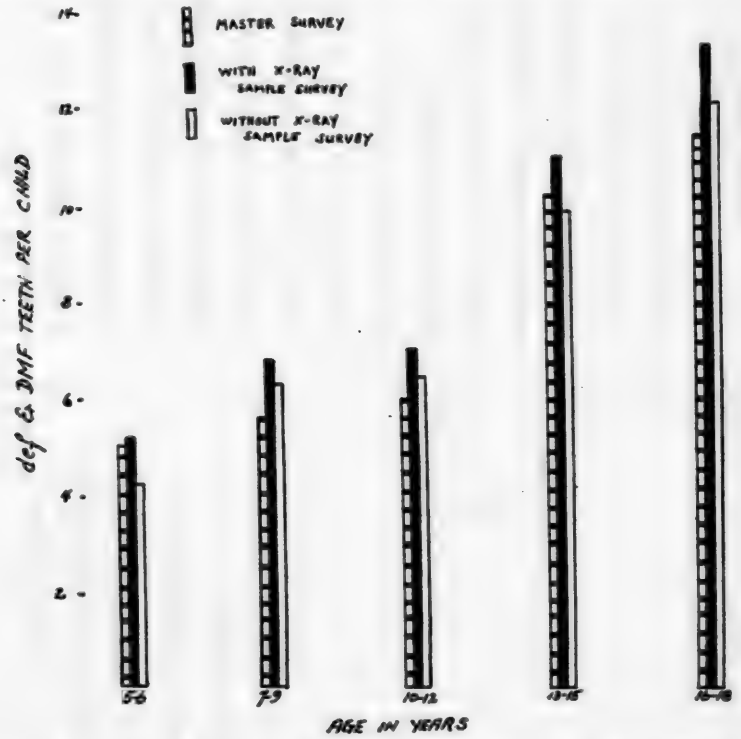

Fig. 5. Average Number of def and DMF Teeth per Child in each Age Group

may readily be influenced by more accurate diagnosis of carious lesions. For that reason, X-ray examination assumes practical importance in public health surveys conducted for dental research purposes.

CONCLUSIONS

1) Dental public health surveys to determine the dental needs of school populations or surveys to compare group dental status, do not require roentgenographic examinations.

2) Dental surveys, conducted for research purposes, require roentgenographic examination for proper interpretation of results.

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CAPACITY OF PERIODONTAL VESSELS ON PRESSURE AND TENSION SIDES OF TEETH MOVED BY ORTHODONTIC APPLIANCES[1]

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Most investigators of the histological effects of movement of teeth by orthodontic appliances have concerned themselves with changes in the bone of the alveolar process and with the behavior of the connective tissue of the periodontal membrane. Little attention has been paid to changes in the periodontal vessels. Several authors have noted that the vessels on the pressure side are markedly reduced in size, but only a few have described specific changes in size, shape or number of these vessels.

Bodecker (1932), in his review of the book by Gottlieb and Orban (1931) on tissue changes resulting from overloading of teeth, stated that there is an initial decrease in the diameter of the vessels on the pressure side, with subsequent increase in diameter in regions of resorptive activity resulting in increased blood supply. On the tension side he reported that the vessels change from "a round to an elliptical form" as a result of the tension. Bodecker's hypothetical figure 6A shows such vessels with their long axes tending to be parallel with the stretched fibers of the periodontal membrane.

Oppenheim (1935) described an "angioma-like" appearance of the vessels on the pressure side, with enlargement and increase in the number of the vessels. Kronfeld (1938) made a similar observation on the pressure side of the periodontal membrane of a human canine. Oppenheim speculated that such a bed of dilated capillaries might serve as "a sort of cushion", a conception previously expressed by Orban (1925) to explain the rich vascularity of the periodontal membrane on the labial side of the rabbit's incisor.

Oppenheim (1942) returned to the subject of these "angioma-like" regions. In reply to Stuteville's (1938) statement that they are spaces caused by autolysis of necrotic connective tissue, by tearing, and by shrinkage during preparation, he maintained that they are endothelially lined spaces containing some red blood corpuscles.

Lundquist (1937) concluded that "pressure seems to determine an augmentation of the circulation", and claimed that the vessels of the pressure side are circular in cross-section due to relaxation of the periodontal fibers. On the tension side, by contrast, he found the vessels oval in cross-section.

[1] Read at Twenty-fourth General Meeting of the International Association for Dental Research, Kansas City, March 17, 1946. (*J. D. Res.*, **25**: 170, 1946.) Received for publication November 8, 1946.

In view of the paucity of definite information concerning the behavior of the vessels when the periodontal membrane is compressed or stretched, this investigation was made. It has, as its primary aim, the determination of a quantitative expression for the number and capacity of the periodontal blood vessels as they are affected by the forces of orthodontic appliances.

Our material included:

1. The upper right and left canines of a 10-year old female macaque moved buccally for about 6 weeks by an orthodontic appliance consisting of lingual expansion wire soldered to bands on both canines.

2. The lower left first and second permanent molars moved distally by the action of a separating wire placed for 1 week between the second premolar and the first permanent molar.

3. The upper right and left first premolars and the lower second incisors, which were not moved, were used as controls.

A block was cut from the upper jaw containing the canines and first premolars of both sides. Two blocks were excised from the lower jaw, one containing the molar region, the other all the incisors. These were fixed in 10% formol, decalcified in 5% nitric acid, imbedded in low viscosity nitrocellulose, sectioned serially, and stained in hematoxylin and eosin. The maxillary block was sectioned first in a nearly frontal plane through the canines to include the greatest length possible of these curved teeth (*fig. 1*). Then, after reorientation, sections were cut in similar fashion through as much as possible of the buccal and lingual roots of the first premolars (*fig. 3*). The mandibular block containing the molars was cut mesiodistally and included longitudinal sections through the distal half of the second premolar, the first and second molars, and the mesial half of the third molar (*fig. 2*). The block containing the incisors was cut mesiodistally, as nearly as possible parallel with the long axes of the roots.

In order to obtain numerical values for the capacity of the blood vessels in the periodontal membranes we employed weighed paper cut-outs. The procedure was as follows:

1. Sections were selected which passed as nearly as possible through the long axes of the roots of the experimental and control teeth.

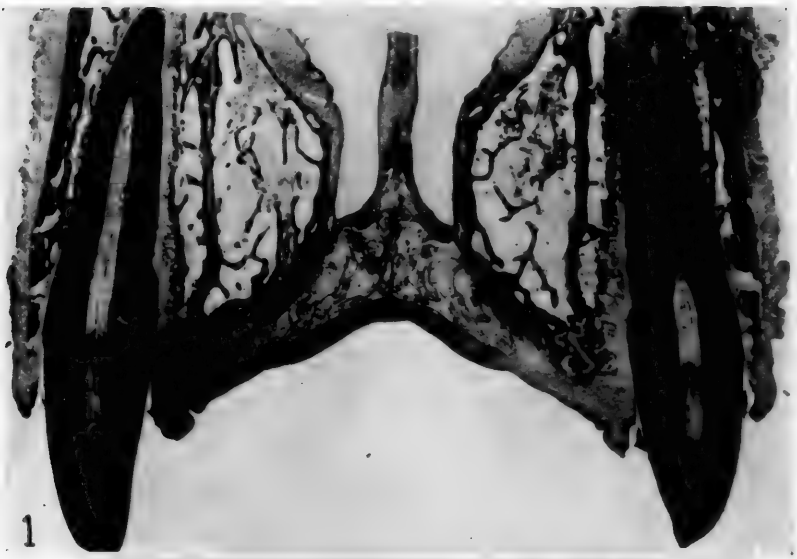
2. Contiguous areas of the sections of the periodontal membranes on the pressure and tension sides were projected onto large sheets of paper at a magnification of 800 diameters.

3. On sketches of these projected images the lumen of each vessel was traced in outline.

4. The outlined successive areas of periodontal membrane were then matched and assembled until the entire area of the periodontal membrane from the level of the alveolar crest to the level of the apex was represented.

5. The vessels entering and leaving the alveolar process were excluded from the areas analyzed by lines arbitrarily drawn between the crests of bone on each side of all vascular foramina.

6. All outlined areas of vessels were then cut out and weighed. The sheets of paper representing the entire area of one side of the periodontal membrane minus the vessels were also weighed (*fig. 4*).



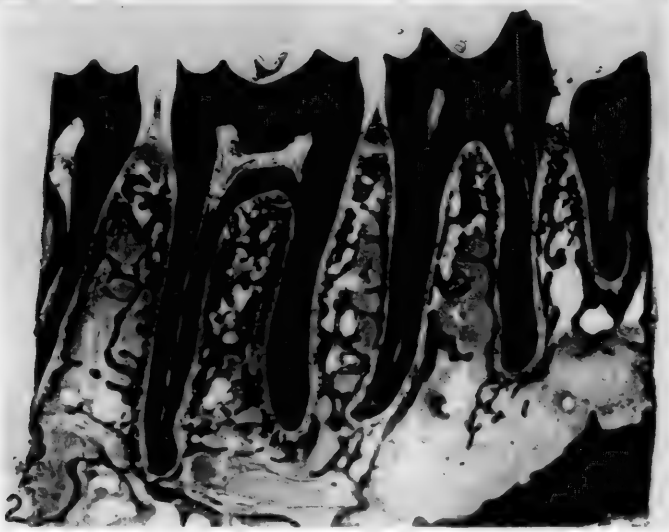


Fig. 1. Frontal section through maxillary canines, moved buccally by lingual expansion appliance. × 5.6 (Orig. mag.)

Fig. 2. Mesiodistal section through (right to left) distal root of lower second premolar, the first and second molars, and mesial root of third molar. Separating wire, between second premolar and first molar, was in place for 1 week. × 5.6 (Orig. mag.)

Since the paper selected was of reasonably uniform thickness and weight, the values so obtained can be regarded as proportional to the volume of the lumina of the vessels and to the volume of the periodontal membrane in which these vessels lie. Hence these values can be used to compare the capacity of the

vessels and the amount of connective tissue on the pressure and tension sides of the periodontal membrane.

Sixteen sections were so analyzed, using in each case an equal length of the periodontal membrane on opposite sides of each root.

For the teeth which were moved, 4 sections of each upper canine and 1 section each of first and second molars were analyzed. For control, 4 sections of upper first premolars and 2 sections of lower second incisors were used.

The data obtained by the foregoing procedure are assembled in parts A & B of Table I. The values entered are the mean weights of the paper cut-outs in grams.

These values are of use only for purposes of comparison. For example, they are used in part C of Table I to express the relation of vascular volume to volume

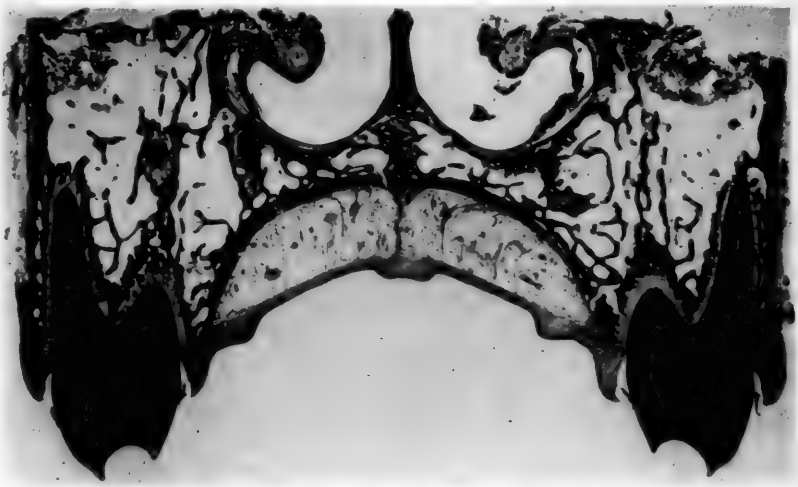


Fig. 3. Frontal section through upper first premolars used as controls. × 5.6 (Orig mag.)

of periodontal membrane on the tension and pressure sides of the teeth studied. In each entry this relation is expressed in the form of the ratio between vascular volume and volume of the periodontal membrane.

In Table II the relation between the vascular volumes of the pressure and tension sides is similarly expressed in the first column. In the second column the entries express the ratio of vascularity of the periodontal connective tissue on the pressure side to that on the tension side. In other words, they show how much richer the blood supply is on the tension side. In the third column the ratios express the difference in periodontal volume on the opposite sides.

These ratios in Table II facilitate the comparison of the effect of orthodontic forces on the blood supply of the periodontal membranes of individual teeth. The canines were moved by the fairly strong, continuous force of a lingual expansion appliance. The vessels of the tension side of the left canine have 6.6

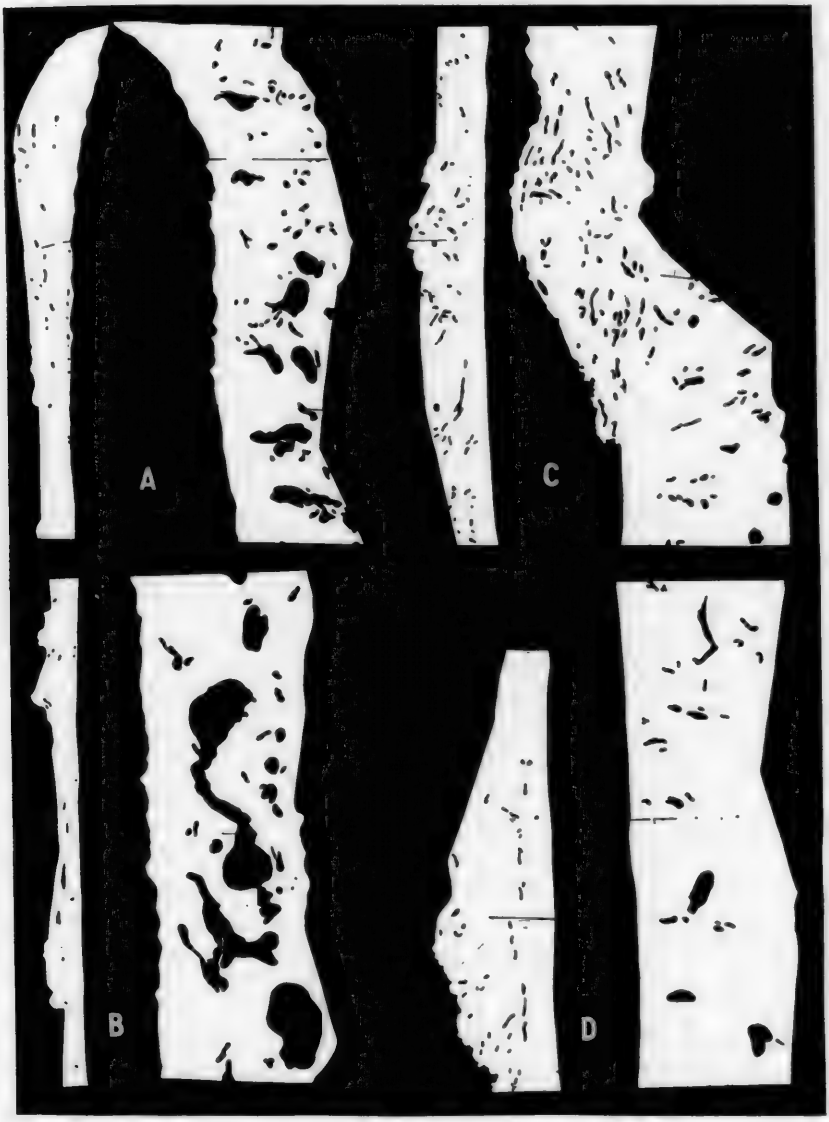


Fig. 4. Cut-outs of representative levels of the periodontal membrane of the upper left canine. A. Apical tenth. B. Near junction of middle and apical thirds. C. At level of resorption of the root shown in *Fig. 1*. D. At alveolar crest. × 52 (original × 800).

times the capacity of those on the pressure side. These vessels lie in a periodontal membrane which has only 1.8 times the volume of that on the pressure side. For the right canine the capacity of the vessels on the tension side is 17.5 times that on the pressure side, in a periodontal membrane which is 2.7 times larger.

From this evidence it may be surmised that the reaction to the force of the appliance was greater on the right than on the left side.

TABLE I

Capacity of vessels and volume of periodontal membrane on opposite sides of roots

	A		B		C	
	Capacity of vessels		Volume of periodontal membrane		Ratio of vessels to periodontal membrane	
	Tension side	Pressure side	Tension side	Pressure side	Tension side	Pressure side
Upper left canine	24.5	3.7	340.1	188.1	1:13.9	1:50.6
Upper right canine	34.9	2.0	329.3	120.0	1: 9.4	1:59.4
Lower first molar	41.7	9.2	145.5	83.8	1: 3.5	1:10.3
Lower second molar	20.3	14.9	178.8	149.4	1: 8.8	1:10.0
	Lingual side	Buccal side	Lingual side	Buccal side	Lingual side	Buccal side
Premolar controls	14.1	11.9	82.9	91.1	1: 5.9	1: 7.6
	Distal side	Mesial side	Distal side	Mesial side	Distal side	Mesial side
Incisor controls	18.4	18.0	89.1	102.7	1: 4.9	1: 5.7

TABLE II

Relative blood supply and amount of connective tissue on opposite sides of the periodontal membranes

TEETH MOVED BY APPLIANCES	CAPACITY OF VESSELS	BLOOD SUPPLY PER UNIT VOLUME OF CONNECTIVE TISSUE	VOLUME OF PERIODONTAL MEMBRANE
	Ratio of pressure side to tension side	Ratio of pressure side to tension side	Ratio of pressure side to tension side
Upper left canine	1: 6.6	1:3.7	1:1.8
Upper right canine	1:17.5	1:6.3	1:2.7
Lower first molar	1: 4.5	1:3.0	1:1.7
Lower second molar	1: 1.4	1:1.1	1:1.2
TEETH NOT MOVED BY APPLIANCES	Ratio of buccal side to lingual side	Ratio of buccal side to lingual side	Ratio of buccal side to lingual side
Premolar controls	1: 1.2	1:1.3	1:0.9
	Ratio of mesial side to distal side	Ratio of mesial side to distal side	Ratio of mesial side to distal side
Incisor controls	1: 1.0	1:1.2	1:0.9

Perhaps a better way of visualizing the relation of the capacity of the vessels on the opposite sides of a root is to determine the amount of connective tissue supplied by the vessels. This can be done by expressing the ratio of the volume of the vessels to the volume of the periodontal membrane. For the upper left

canine the volume of the blood in the vessels of the tension side supplies 13.9 times this volume of connective tissue (Table I, column C), whereas on the pressure side the volume of connective tissue is 50.6 times that of the blood. That is, the blood supply is 3.7 times richer on the tension side (Table II, second column). For the right canine the vascularity on the tension side is greater (1:9.4), but poorer (1:59.4) on the pressure side. Here the vascularity on the tension side is 6.3 times richer than on the pressure side.

Similar comparison was made of the values for the lower first and second molars, moved by the force of a separating wire between the molar series and the second premolar. As *fig. 2* and the entries in Table I, column B show, the mesial membranes of these teeth are noticeably thicker than those on the distal side. This demonstrates that some distal movement was induced, since the effect of normal mesial drift would be to produce thinner membranes on the mesial sides. The vascularity on the tension side of the first molar is nearly 3 times richer than that for the second molar (3.1 and 1.1 respectively in Table II, second column). This we take as evidence that the force exerted by the separating wire was expended chiefly on the first molar and had but little effect on the second molar.

Since the premolars and incisors used for controls had no planned pressure and tension sides, the buccal and lingual sides were compared for the premolars and the mesial and distal sides for the incisors. For both types of control teeth all the ratios expressed in Table II approach equality in about the same degree as the ratios for the second molar, a fact which can be interpreted as evidence that these teeth were not subjected to abnormal forces.

It is generally accepted that osteogenesis occurs in regions of relative ischemia; osteoclasis in regions of relative hyperemia (Leriche and Policard, 1928). At first glance our findings would seem to contradict this rule, since there is active formation of new bone on the tension side where the blood supply is rich and active resorption of bone on the pressure side where the blood supply is poor. This apparent discrepancy is explained by the fact that most of the increase in volume of blood on the tension side is due to increased size of the longitudinal periodontal vessels, the capillaries being sparse in the immediate vicinity of regions of bone formation. On the pressure side the longitudinal vessels are much reduced in caliber, but there is a rich bed of capillaries in the immediate vicinity of each region of bone resorption.

This marked increase in number of capillaries in regions of resorption explains the fact that, though many of the vessels on the pressure side had their lumina completely obliterated and were not counted, the total number of vessels showing lumina on the pressure side is approximately equal to that on the tension side. If the additional capillaries in resorptive regions are ignored, the number of vessels with open lumina is markedly less on the pressure side.

Where the vessels on the pressure side show definite lumina there is no change in shape, the vessels remaining cylindrical, as they are also on the tension side. This is contrary to the observations of Bodecker and Lundquist. It is our opinion that the "oval cross-sections" of Lundquist and the "elliptical form" of Bodecker are simply oblique sections through cylindrical vessels.

In none of our sections do we find the "angioma-like" appearance described by Oppenheim and Kronfeld. The only regions which might be interpreted as approximating such a condition are those in which active resorption is in progress, with the marked increase in number of capillaries mentioned above. This does not, however, exceed the normal increase in regions of active osteoclasis.

SUMMARY

In a 10-year old macaque the method of weighed paper cut-outs was used to compare the vascularity of the periodontal membrane on pressure and tension sides of teeth moved by orthodontic appliances. Though the number of vessels on opposite sides is approximately equal, the vascularity of the tension side is greater than that on the pressure side in proportion to the force applied. For example, for the upper canines moved by the fairly strong continuous force of a lingual expansion arch wire the tension sides of the periodontal membranes have respectively 6.3 and 3.7 times richer blood supply than the pressure sides. A lower first molar, subjected to the brief action of a separating wire, has 3.0 times richer blood supply on its tension side. The vascularity of opposite sides of the periodontal membranes approaches equality in the adjoining lower second molar and in incisors and premolars used as controls. In our serial sections the vessels on the tension side are markedly larger than those on the pressure side, but all vessels retain their cylindrical form. The equality of number of vessels with open lumina on opposite sides is due to greatly increased numbers of capillaries in regions of bone resorption.

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EXPERIMENTAL AMELOTOMY[1]

Report of Ground Section Study

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The separation of tooth germ elements has shed some light on the phenomena associated with the calcification of enamel. A previous report (1) described the results of the surgical removal of the dental pulp of unerupted cats' teeth before complete formation of the enamel. It was found that the enamel remained in the matrix state. A tentative conclusion was drawn that the dental pulp was essential to the calcification of the enamel. The results further suggested that the calcification of enamel beyond the matrix state was a continuous process dependent upon the pulp, including the odontoblasts.

This report deals with another phase of the investigation in which the enamel organ of incompletely formed teeth of dogs was partially removed. The previously reported experimental procedure in pulpectomy was also repeated on dogs. At the present time, macroscopic observation and ground section evidence seem to verify the previous conclusions.

The experiments were performed on 9-week old dogs. The state of tooth development is comparable to that of the cats used in the pulpectomy experiment (1). An examination of the degree and pattern of calcification at the time of the operation is important, hence control specimens of the calcification phenomena were examined in ground and decalcified sections.

Fig. 1 is a photomicrograph of a decalcified section of a permanent lower third incisor of a 61 day old dog. The enamel (E) is acid resistant and incompletely formed. This section is included to prove that the enamel at this stage of development is in the acid resistant state. *Fig. 2* is a ground section of the opposite incisor of the same animal. The tooth was removed from its bony crypt and fixed in 10% neutral formalin. The specimen was prepared on ground glass with pumice and polished on an Arkansas stone so as to obtain a 180 micron thick section. The enamel is in the matrix state and exceedingly friable, causing many cracks in the direction of the enamel rods and some loss at the

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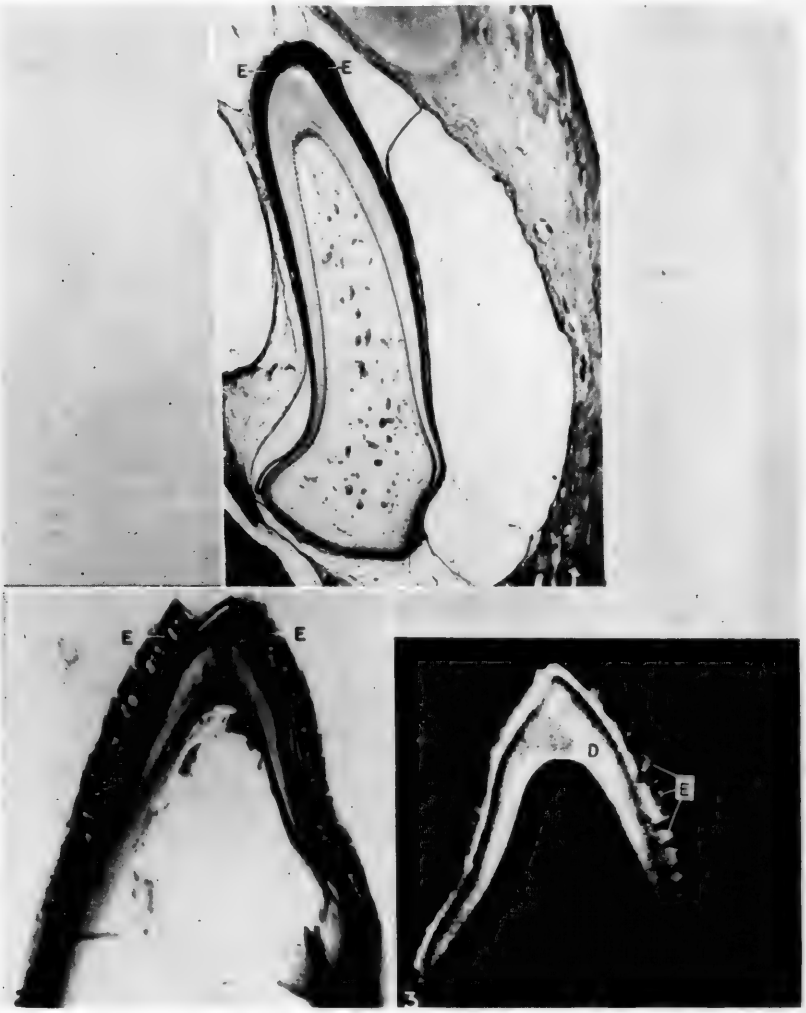

Fig. 1. Photomicrograph of decalcified section of permanent lower third incisor of 61 day old dog. Enamel (E) acid resistant and incompletely formed. H & E (21× Orig. mag.)

Fig. 2. Photomicrograph of ground section of permanent lower third incisor of 61 day old dog. Loss of enamel at incisal and cracks due to friability of acid resistant enamel. (45× Orig. mag.)

Fig. 3. Enlarged photograph of soft x-ray shown in *fig. 2.* Enamel (E) more highly calcified at dento-enamel junction diminishing toward periphery. Dentin (D). (45× Orig. mag.)

tip. *Fig. 3* is an enlarged photograph of a soft x-ray of the specimen shown in *fig. 2.* The enamel (E) shows variations in calcification. The first formed increments are more highly calcified than the last, indicating that, during formation in the matrix state, the calcification proceeds from within outward similar

to the pattern of matrix formation. The mineral poor peripheral layer of dentin is exceptionally marked at this stage. Other parts of the dentin appear to be as highly calcified as the innermost increments of enamel.

Noyes, Schour and Noyes (2) established a research trend by separating calcification from formation and described them as 2 separate processes. Investigators since this time have accepted, that calcification relates only to the deposition of calcareous matter in an organic matrix, a distinction that aids in the comprehension of enamel calcification.

Some investigations of enamel in the matrix or acid resistant state are assumptive and leave the impression that, in this state, the enamel is a homogeneous tissue. The chemical analysis of acid resistant enamel (3) does not explain the progressive nature of its calcification.

The general pattern of calcification in the matrix state of all calcified tissues (enamel, dentin, cementum and bone) is that of incremental growth, in that, an uncalcified zone remains until calcification is complete. It is a generally known fact that calcification always occurs farthest away from the source and then proceeds towards the origin of the calcific material; enamel calcification is no exception. When finally the last increment is calcified, the crown is completed. Noyes, Schour and Noyes (4) refer to it as incremental calcification. Weinman, Wessinger and Reed (3) reported that at this state the enamel is 35% calcified.

The soft x-ray examination of enamel in the matrix state is in keeping with the recent investigations of amelogenesis. Two groups of authors (5, 6) who recently reported on this subject, found that mineral salts were deposited in the already formed organic matrix. This occurs shortly after formation begins and continues until the complete enamel cap is formed.

Unless the problems of formation and calcification of enamel in the matrix state are understood, erroneous concepts may follow. The concept that enamel in the matrix state is a homogeneous tissue, grew out of the method of examining it *en masse* to determine its physical and chemical characteristics. Furthermore, the use of decalcified sections to study the calcification of enamel lends itself to error. The decalcified matrix stains rather homogeneously, even though it is more calcified in the innermost layers.

Diamond and Weinman (7) stated "According to our present concepts therefore, *the term calcification should be applied only to the so-called maturation state in the formation of enamel.* We are now ready to present additional evidence *that this final maturation stage which we now feel should be called calcification does not begin at the dentino-enamel junction, to proceed outwardly in the direction of the periphery, parallel to the direction of the incremental lines.* (Italics in original.) These authors have excluded an important phase of enamel calcification. The soft x-ray studies (*fig. 3*) corroborate the findings of Noyes, Schour and Noyes (4); Nuckols, Saunders and Frisbie (5); Orban and Sicher (6). These investigators all agree that enamel is laid down first as an organic matrix and that mineral salts are deposited therein. This process must be included in the consideration of enamel calcification.

The incremental pattern of calcification in the matrix state suggests that the source of calcification is the enamel organ. Studies by Bevelander (8) verify this hypothesis. However, it is definitely established that, following the reduction of the enamel organ, the calcification continues. Chase (9) estimated that mature enamel requires 3 months beyond the matrix state for complete calcification. It is hardly conceivable that the reduced enamel epithelium can perform this function. Its function has been described by Gottleib, Orban and Diamond (10) as a protective mechanism against resorption prior to eruption.

The suggestion that the dental pulp may possess the function of calcifying the enamel is not new in dental literature (11, 12, 13). The authors have published experimental evidence to support this thesis (1). Previously, they attacked the problem from the viewpoint of the pulp; its removal resulted in a failure of post-matrix calcification of the enamel. The present report deals with the surgical removal of a part of the enamel organ. Examination of the results of ground sections indicates that the calcification of enamel in the operated area proceeds to completion.

TECHNIQUE OF EXPERIMENT

An incision was made in the floor of the mouth over the lower right third incisor. The bony crypt was exposed surgically and the enamel organ was removed from the incisal half of the crown. The surface of the enamel was gently scraped to insure removal of the ameloblasts. The tooth was tilted slightly to accomplish this end. The incision was sutured and the animals had an uneventful recovery. The formation of the tooth at the time of operation is comparable to that shown in *figs. 1*, *2*, and *3*.

In order to determine the efficacy of the experiment, 1 animal was sacrificed immediately after the operation (*fig. 4*). The opening into the bony crypt was made at O. The tooth was tilted into this opening and the enamel organ removed from A to A′. The remainder of the enamel organ, as well as Hertwig's sheath was intact. The loss of enamel, particularly on the lingual surface was caused in the effort to remove all the enamel organ during the operation.

Fig. 5 is a high magnification of area "I" in *fig. 4* which demonstrates the complete removal of the enamel epithelium in this zone. A small hemorrhage may be seen at "H". *Fig. 6*, a high power photomicrograph of area 2, *fig. 4* shows normal epithelium. The separation from the follicular wall may be the result of trauma.

A further check was made on trauma by examining a specimen taken 2 weeks following amelotomy. *Fig. 7* is a photomicrograph of a lower right third incisor which had been operated 2 weeks previously. The tissues appear normal. No enamel epithelium is present in the operated area and no traumatic influence of the surgical intervention is evident. The surgical opening (O) is almost completely repaired with newly formed bone. *Fig. 8* is a higher magnification of the incisal area of *fig. 7*. The acid resistant enamel is in contact with the follicular wall in which a few capillaries appear. The enamel rods are cut transversely in the peripheral zone (Ex) and the roughness on the surface is undoubtedly due to the operative technique.

X-ray examination of the area showed a malposition of the operated tooth and retarded eruption. *Fig. 9* is a photograph of the mouth of a dog 24 weeks after amelotomy. The animal was operated at 61 days of age. The lower second incisor occupied a position in the arch between the canine and the first incisor. The experimental tooth (arrow) erupted in the floor of the mouth lingual to the canine. This malposition was observed in all amelotomized teeth. The malposition of the experimented tooth was not due to the surgical opening made in the mandible. The 2-weeks specimen (*fig. 7*) demonstrates that complete bone repair had occurred.

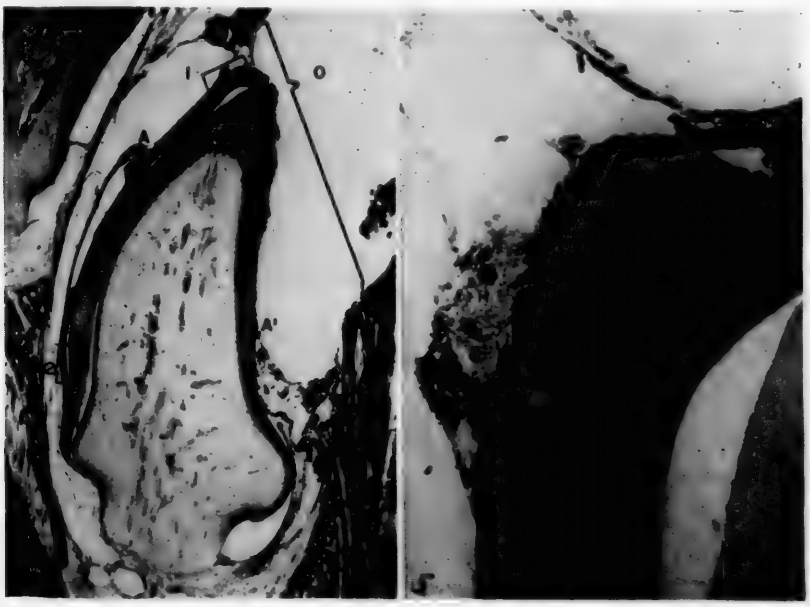

Fig. 4. Photomicrograph of decalcified section of immediate post-operative specimen of 61 day old dog. Surgical opening in mandible (O). Enamel organ removed from A-A' including much of the matrix on the lingual surface. Enamel acid resistant. Note loss of enamel due to operative procedure. H&E (21× Orig. mag.)

Fig. 5. Higher magnification area I *fig. 4*. Enamel organ completely removed. Area of hemorrhage (H) Enamel (E) Dentin (D).

Macroscopic examination of the experimental tooth showed a slightly roughened surface of hard enamel which appeared calcified. The incisal portion was deficient in thickness of enamel formation, designating the area of removal of the enamel organ. Apparently the formation was not complete at the time of operation and no further enamel matrix could form in the absence of the enamel epithelium.

Fig. 10 illustrates the macroscopic picture of calcification of the amelotomized tooth (Ex) which shows a hard glistening surface reflecting the light almost as brightly as does the enamel of the canine tooth (C'). The slight pitting of the enamel surface of the experimental tooth probably is due to the absence

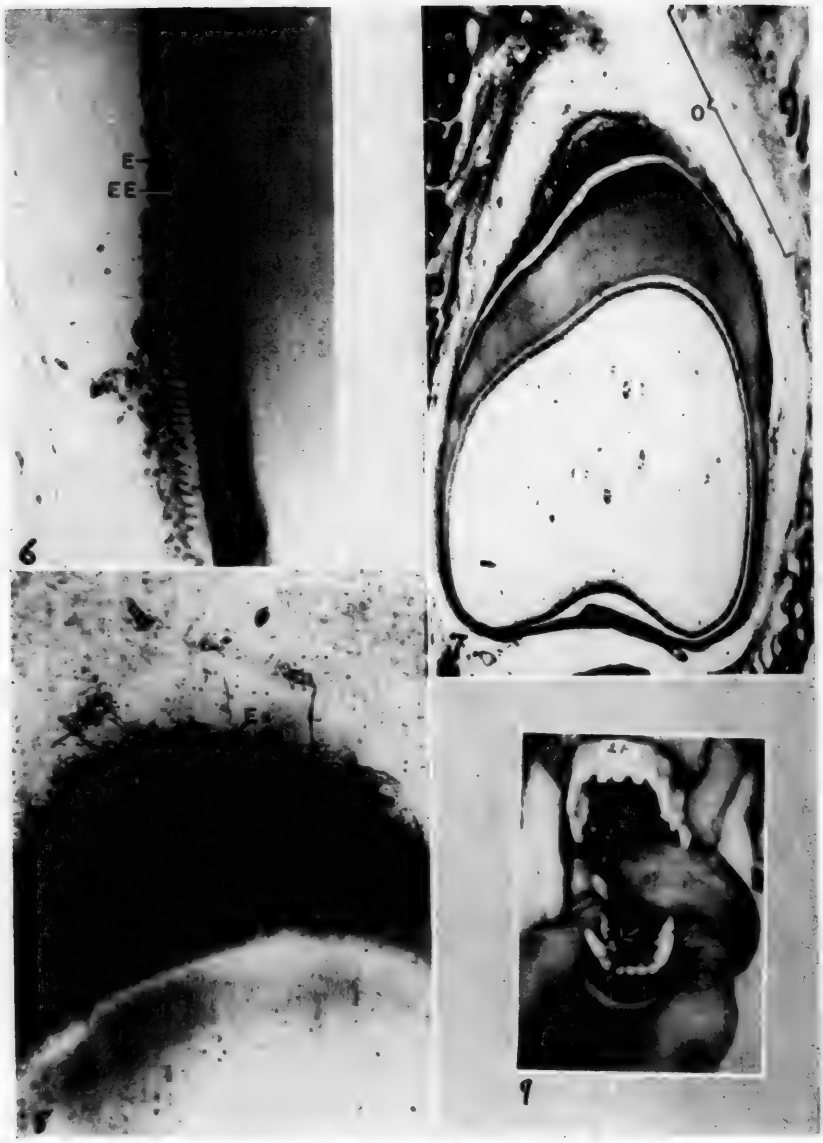

Fig. 6. Higher magnification area (2) *fig. 4* showing normal enamel epithelium (EE) Enamel (E) Dentin (D).

Fig. 7. Photomicrograph of permanent lower third incisor of dog 2 weeks after amelotomy. Surgical opening (O) into bony crypt repaired with bone. Enamel epithelium not present in operated zone. Enamel acid resistant. H & E (21× Orig. mag.)

Fig. 8. Higher magnification of incisal area *fig. 7.* Enamel (E) in matrix state. Peripheral rods cut transversely. Enamel in contact with follicular wall. No enamel epithelium present.

Fig. 9. Photograph mouth of dog 24 weeks after amelotomy performed at age 61 days. Note malposition (arrow) of experimented tooth hypoplastic in incisal portion. Cervical portion completely formed.

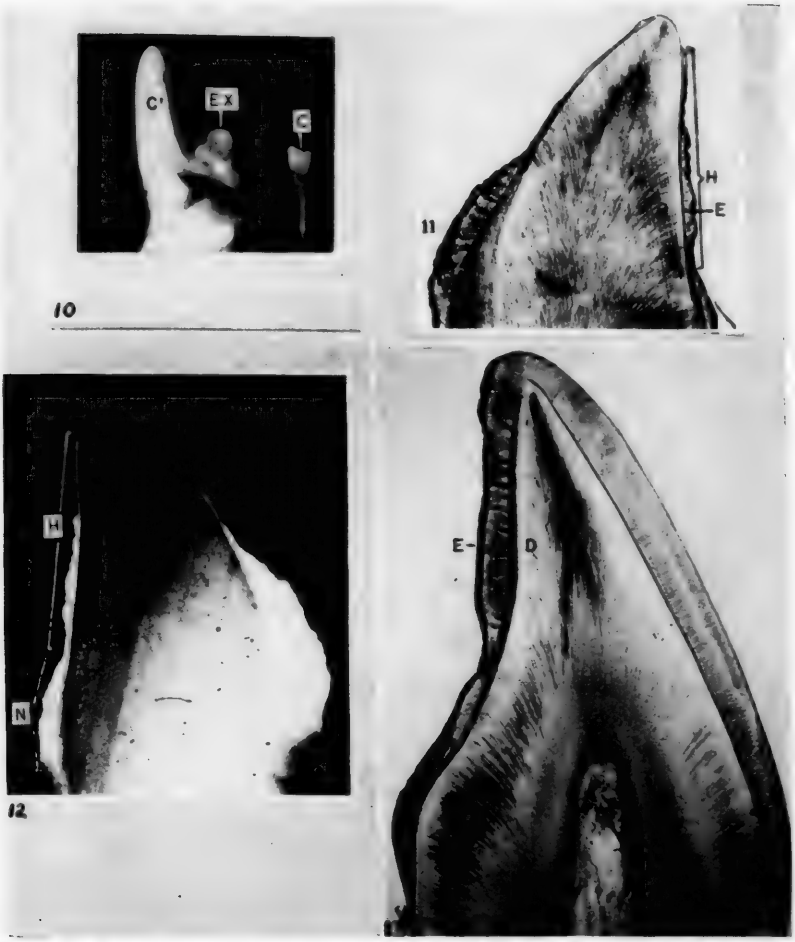

Fig. 10. Higher magnification of specimen shown in *fig. 9*. Experimental tooth (EX) demonstrates clinical appearance of hypoplasia and normal calcification. Canine (C') normally calcified. Tooth mounted on pin (C) demonstrates appearance of specimen at time of amelotomy.

Fig. 11. Photomicrograph of ground section of amelotomized tooth shown in *figs. 9* and *10*. Some enamel lost in preparation of section. Area of removed enamel organ demonstrates hypoplasia (H). Enamel E. (25× Orig. mag.)

Fig. 12. Enlarged photograph of soft x-ray of section shown in *fig. 11*. Area of hypoplasia (H) induced by amelotomy as well calcified as normal enamel (N). (photograph reversed)

Fig. 13. Photomicrograph of ground section of control tooth (opposite side) for specimen *fig. 10*. Note complete formation of enamel E. Dentin D. (20× Orig. mag.)

of its enamel cuticle. Near the cervix of this tooth, there is a thickening of the enamel due to the continued formation of enamel matrix by the intact enamel organ. This difference in thickness, in comparison to that of the incisal portion is proof that the removal of the enamel organ was complete in the latter area.

The tooth mounted on a pin (*fig. 10*, C) serves as a control; it was removed from another dog of the same age and shows the condition of the amelotomized tooth at the time of the operation. This control tooth was subsequently sectioned and photographed as *figs. 2* and *3*. Its surface appears somewhat like unglazed porcelain and only one incisal corner (white) is more highly calcified than the rest of the crown. Both experimental and control teeth were stained with safranin to determine their relative permeability. The control tooth (C), taking up a great deal of dye, was stained a deep red, particularly near the least calcified cervical area, while the experimental tooth (Ex) showed only a slight stain on its pitted surface.

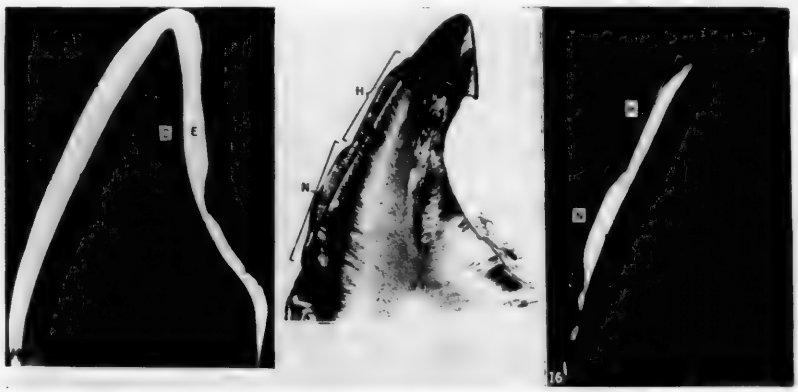

Fig. 14. Enlarged photograph of soft x-ray of control shown in *fig. 13*. Note complete calcification. (photograph reversed)

Fig. 15. Photomicrograph of ground section of amelotomized tooth. Specimen taken 31 weeks after operation. All but labial enamel lost in preparation of section. Operated area shows hypoplasia (H). Normal enamel (N). (20× Orig. mag.)

Fig. 16. Enlarged photograph of soft x-ray of specimen shown in *fig. 15*. Operated area showing hypoplasia (H) of enamel. Normal enamel (N). Note complete calcification of amelotomized area.

Ground sections were made of 2 amelotomized teeth while the homologous teeth from the opposite side served as controls. *Fig. 11* is a ground section of the specimen shown in *figs. 9* and *10*. The loss of much of the enamel appears to be due to the grinding technique. The area of induced localized hypoplasia (H) caused by the amelotomy is evidence of failure of complete formation in the incisal half. A soft x-ray of the same specimen (*fig. 12*) shows that calcification of the experimental area (H) is equivalent to the normal area (N) at the cervix. Compared with the opposite tooth as a control (*fig. 13*) both photomicrographs and Grenz ray (*fig. 14*) show completion of anatomic form and calcification.

A second experimental tooth (*fig. 15*) was operated at 61 days of age. The specimen was taken 31 weeks later. Much of the enamel in this case was lost from the lingual surface in operative procedure. However, more enamel re-

mained on the labial surface. The limit of the operation may be seen by the extent of induced localized hypoplasia (H). The remaining normal enamel (N) continued to form. The soft x-ray (*fig. 16*) shows the quality of calcification of the normal and experimental areas (H).

DISCUSSION

A discussion of the calcification of the enamel cannot be made without reviewing the earlier reports of the authors on pulpectomy (1). This investigation showed that in the absence of the dental pulp and odontoblasts, enamel calcification did not pass beyond the matrix state. The enamel organ degenerated into squamous epithelium and underwent cystic degeneration. It may be construed that if the enamel organ were responsible for the maturation of enamel, then the degeneration of this tissue might be associated with the failure of post

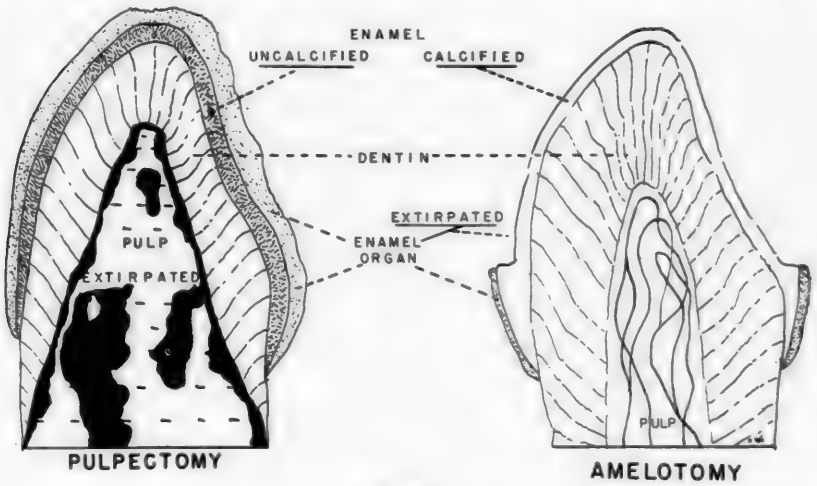

Fig. 17

matrix calcification. With this thought in mind the investigation in amelotomy was conceived.

The removal of a part of the enamel organ resulted in the incomplete formation of the enamel in this area, as matrix formation came to a standstill. The hypoplasia was not of systemic origin, because other teeth at the same period of formation were perfectly formed. It is evident that the enamel organ was removed before complete formation of the incisal half of the tooth.

The calcification of the amelotomized area is equivalent to the normal control area of the same tooth at the cervix as well as the control tooth on the opposite side. This evidence demonstrates that post-matrix calcification occurred in the absence of the enamel organ.

The pattern of post-matrix calcification (10) conforms to the recognized phenomena of calcification in which this process begins at the most remote area and proceeds toward its source, the pulp.

The fact that post-matrix calcification did not occur in pulpectomy and did occur following amelotomy is offered as evidence that the dental pulp is responsible for maturation of enamel. This concept is in keeping with the hypothesis that enamel maturation is a continuous process associated with the activity of the pulp. *Fig. 17* is a diagram illustrating the two different results of amelotomy and pulpectomy.

It is interesting to point out that a situation similar to pulpectomy may be found in hereditary opalescent dentin. The pulp in this condition contains very few odontoblasts and the enamel and dentin are very poorly calcified. Cases under observation by the authors show complete attrition of the crowns of the first molars at the age of 12. It was suggested by the authors in their previous publication that the odontoblasts were responsible for the calcification of the enamel. The absence of odontoblasts in hereditary opalescent dentin suggests that these cells are concerned with the calcification of both the enamel and dentin.

The authors wish to withhold definite conclusions until the examination of decalcified sections is completed. The sections are in progress and the report will be made shortly.

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INDUCED FUSO-SPIROCHETAL INFECTION IN DOGS[1]

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The number of unsuccessful attempts to produce fuso-spirochetal infection in animals by the injection of only the organisms taken from lesions and cultures suggests that some other factor or factors are necessary to produce the disease in experimental animals (1, 2). Kline (3), was able to produce gangrenous lesions in the groin of guinea pigs with the injection of fuso-spirochetal organisms only after the tissues had been traumatized. D. T. Smith (4), produced noma in guinea pigs that had been fed a scorbutic diet for 3 weeks and then were inoculated in the mucous membrane of the cheek with fuso-spirochetal organisms. He also injected various mixtures and combinations of organisms to produce abscesses in the groin of guinea pigs. The organisms that he selected were anaerobic hemolytic streptococcus, treponema microdentium, the small fusiform bacillus and vibrio viridans. His conclusions were that the etiological agent in fuso-spirochetal lesions was a probable symbiosis of a spirochete, a vibrio, a coccus, and a fusiform bacillus, but not necessarily the organisms that he had used. Wallace, Wallace and Robertson (5), reported that daily sublethal intravenous injections of Scillaren B resulted in the appearance of ulcerative stomatitis and bacterial studies from the lesions showed a great number of fusiform bacilli and spirochetes. Tunnicliff and Klein (6), using the Scillaren B on guinea pigs, found that it produced a leucopenia and dilatation of the blood vessels of the skin. The injection of fusiform bacillus into these prepared areas produced abscesses from which the bacillus and spirilla-like organisms were demonstrated in smears and sections. In summarizing a review of the literature, it may be said that when attempts to induce experimental fuso-spirochetal infection in animals have been successful there had been a lowered tissue resistance in the animal or various combinations of organisms had been inoculated.

The method used to produce fuso-spirochetal infection in this study was to lower the tissue resistance in dogs. This was accomplished by daily intravenous injections of Scillaren B. Scillaren B is an amorphous glucoside occurring in squill and has pharmacologic properties similar to those of digitalis, though it is less persistent but similarly cumulative. It also may be used as a diuretic and expectorant.

After various dosages of the drug had been used, it was found that 0.08 to 0.10 mgm per kilogram would develop fuso-spirochetal lesions in the dogs. The

[1] Read at the Twenty-second General Meeting of the International Association for Dental Research at Chicago, Illinois, March 18, 1944 (*J. D. Res.*, **23:** 190, 1944). Received for publication February 18, 1947.

first reaction observed was that the heart rate decreased to 100–105 per minute, a drop of 10 to 12%, a few minutes after the injection, but the beat became much stronger. Within 15 minutes from the time of the injection, a regurgitation would usually occur. It may be only mentioned here that there is a diversity of opinion concerning the cause of this regurgitation. Some believe that it is a vagal reflex, while others believe it is due to a direct action on the vomiting center.[2]

No other reaction could be seen on the first day. After the second injection the following day, the dog showed signs of lassitude, loss of appetite, excessive salivation and the usual regurgitation. These symptoms became progressively accentuated each day, causing extreme debilitation of the animal.

Smears were taken from the gingival sulcus and tooth surface of the dogs before the injections were started. These were stained with methylene blue and examined for fusiform bacilli and the spirochete. Without exception all the dogs examined showed these organisms. It was noticed that the number of these organisms increased after the third or fourth injection. Concurrent with this, a dark brown-colored debris appeared on the posterior teeth. The gingival margins became acutely inflamed, had a very foul odor, and bled on the slightest provocation.

On the fifth or sixth day, there appeared on the mucous membrane, areas of hyperemia. They were usually located on the buccal mucosa adjacent to the posterior teeth and on the gingival tissues. The average measurement of these areas were between 15 to 20 millimeters in width. Later the surface became granular and soon after, the lesions ulcerated. With their raised and undermined edges their appearance was similar to a primary chancre of syphilis. However, these lesions apparently were more painful than a chancre as was evident when they were touched with an instrument. Smears from these ulcerations showed many spirochetes and fusiform bacilli. The dogs at this time were very weak and debilitated and would die within a day or two if the injections were continued.

Attempts were made to transmit the infection into guinea pigs. A suspension of organisms was made from material obtained from a dog in the later stages of the disease and injected into the groin of the guinea pigs. There was a slight inflammatory reaction as would be expected but no abscess formation was observed. Another guinea pig was injected in the groin with a 0.15 mgm.

[2] The nausea and emesis are not due to local gastric irritation as was formerly thought. They set in only when the cardiac effects are manifest, and are best explained on the assumption that the emetic center is stimulated by reflexes set up by the cardiac action; in other words, the emesis seems to be secondary manifestation of the cardiac action.

The small importance of local irritation is indicated by the fact that emesis occurs more readily with intravenous than with oral administration, and vomiting movements take place when the whole alimentary canal has been excised (Sollmann). Nor is the action directly on the vomiting center, for if the brain is artificially perfused, emesis results from injection into the femoral vein, from where it cannot reach the brain. Emesis does not result if oubain is added to the fluid that perfuses the brain, but does not reach the general circulation. Moreover emesis is prevented if the spinal cord is divided at the level of the second dorsal vertebra.

of Scillaren B and a few hours later it was injected in the same area with the same suspension of fuso-spirochetal organisms. In 48 hours an abscess had formed

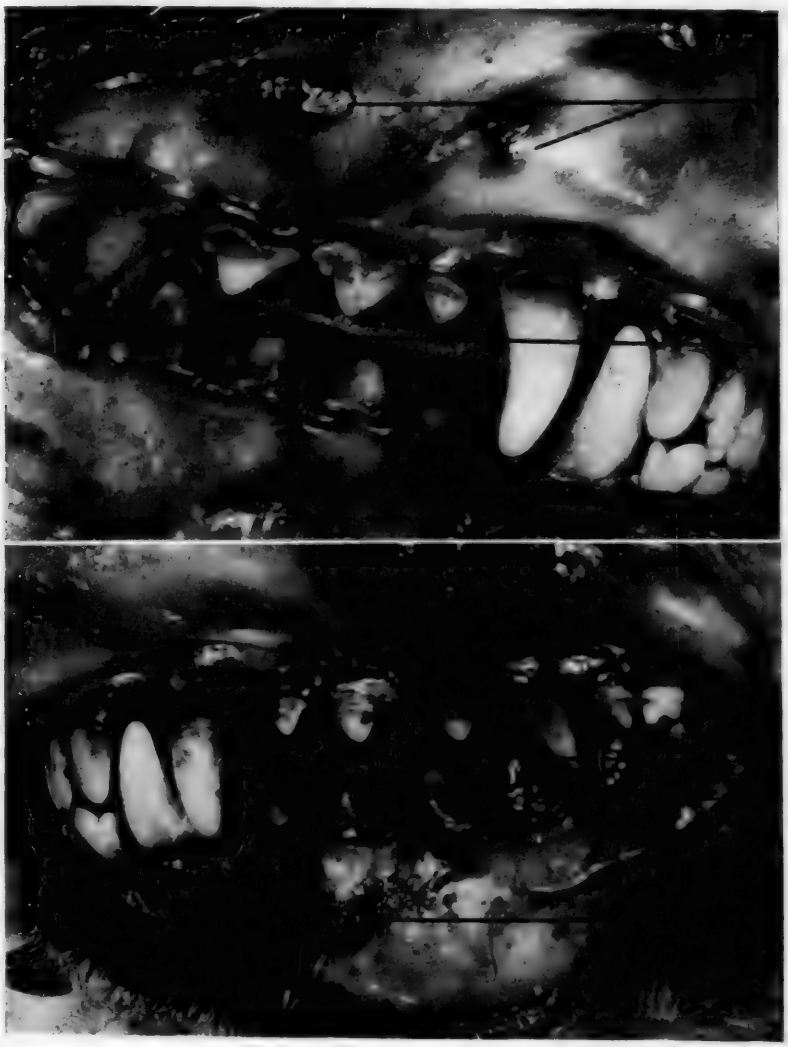

Fig. 1 Photographs of a dog that had been injected with 0.08% mgm. of Scillaren B per kilogram for 10 days. (*a*) Ulceration in muco-buccal fold. (*b*) Excessive debris on teeth. (*c*) Ulcerations occuring on the tongue. (*d*) Area of inflammation prior to ulceration.

that measured 2 cm. in diameter. The animal was killed and the abscess examined. It was well walled off and contained some thick, yellow, purulent material that had a foul odor. This material was stained with gentian violet

and examined microscopically. An occasional fusiform bacillus and spirochete were found in this examination.

It was reported by Rosenthal (7), that dogs which had saliva containing sodium carbonate would not develop the common lesions of fuso-spirochetal infection. Samples of saliva were taken during the course of the investigation and were analyzed for the carbonate. From our data, we could find no correlation between the amount of sodium carbonate in the saliva and the developing lesions in the mouth.

Red and white blood counts were taken during the course of the injections. As a general rule the erythrocyte count had only a slight variation, which could not be considered pathological. The leucocyte count was more irregular, but generally it was lowered. Tunnicliff and Klein report a decrease of 4,000 in leucocyte counts on guinea pigs after they had been injected with Scillaren B. It is believed that the toxemia produced by the Scillaren B has a depressing effect on the leucocytic forming organs, resulting in a leucopenia.

DISCUSSION

The exact way this drug acts on the tissues to produce fuso-spirochetal infection is not completely understood. It can be accepted that there is probably a lowered resistance of the tissues. This may be brought about by the local irritant action of the drug or by its action on some distant point or a combination of these. The existence of a local lowered tissue resistance was borne out by the formation of abscesses in the groin of guinea pigs.

The active cardiac principle of Scillaren B is similar to that of thevetin, oubain, digitalis, and other digitalis-like drugs and derivatives. All of these drugs have some irritant qualities when injected subcutaneously. Histologic sections of tissue that had been previously injected with Scillaren B showed a dilatation of the capillaries. This suggests that Scillaren B might also have a similar local irritating action.

The infection may not be due to the local action of the drug, but there may be some general reaction which would tend to lower the resistance of the animal. The constant regurgitation and lack of appetite resulted in the loss of approximately one-third of the original weight of the animal, and, therefore, extreme debilitation existed. The defensive mechanism of the blood was also impaired as was shown by a decrease in the leucocyte count. Scillaren B is very toxic in large doses and the resulting toxemia may have depressed the formation of the leucocyte cells. In the later stages of the disease when ulcerations were present in the mucosa, the heart rate had dropped down to less than half the normal rate. The beat was very irregular with extra systoles, the heart apparently being in complete block. It can be assumed, therefore, that the heart was displacing far less than the usual amount of blood per minute, which in turn would cause an anoxic state in the tissue. With the decrease in leucocyte cells and less blood flowing through the tissue at a slower rate in a very debilitated animal, it is understandable that the tissue resistance of the animal would be lowered.

Wallace, Wallace and Robertson found that daily intravenous injections of Scillaren B was the only drug of the cardiac group that produced fuso-spirochetal infection in dogs. This may be due to a strong irritating contaminent present in Scillaren B that is not found in the others that would be conducive to the disease. This is possible since Scillaren B in its pure form is amorphous while the majority of the others in this group are crystalline.

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BILATERAL DENS IN DENTE[1]

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When Kronfeld (1) in 1934 reported his case of dens in dente, and reviewed all the cases previously recorded in dental literature, there were approximately 14 in all, and all were unilateral. Kronfeld's case was the only one with an adequate case history and complete description. He had, however, decalcified his specimen in order to secure serial sections and, therefore, the enamel was lost. Kitchin (2) described a case of dens in dente of the single coronal-invagination type in a "peg-shaped" upper right second incisor, which was ground and shows the continuity of the enamel of the "outer tooth" with that of the "inner tooth." Kitchin mentioned the fact that although the upper left second incisor was shaped like the upper right, the radiograph showed no evidence of dens in dente.

AUTHORS' CASE

Our case is the only one, so far as we can ascertain, of *bilateral* dens in dente. It is from the practice of F. M. McCarthy, Sr., D. D. S., of Olean, New York, who secured the pictures of the patient and the radiographs of the case.

The patient, an 8 year old girl, had a typical parulis, (*fig. 1*), over the apex of the permanent upper left second incisor. This tooth and the permanent upper right second incisor were "peg-shaped." The radiograph (*fig. 2*) shows that each tooth had apical radiolucency, was not fully developed, and had the anomaly, a "tooth within a tooth."

There was no evidence of other oral anomalies. The parents, who were given complete oral examinations show no oral anomalies. Further ancestral history is not available.

The teeth were extracted. *Fig. 3* shows the extracted teeth. *Figs. 4* and *5* show the incisors sectioned longitudinally. On the incisal edge of each tooth along the flattened portion there is a minute choked-up opening that extends into the cavity of the "inner tooth." There was no caries and the enamel was sound and intact in each tooth except for the opening on the incisal edge.

DISCUSSION

Kronfeld (1) stated that a dens in dente may develop, not by actual ingrowth of enamel organ into underlying pulp, but by relative retardation in growth of a portion of the enamel organ, possibly at or near the lingual pit, while the sur-

[1]Presented at the 24th General Meeting of the International Association for Dental Research March 17, 1946, Kansas City, Mo. (*J. D. Res.* **25**: 185, 1946). Received for publication November 13, 1946.

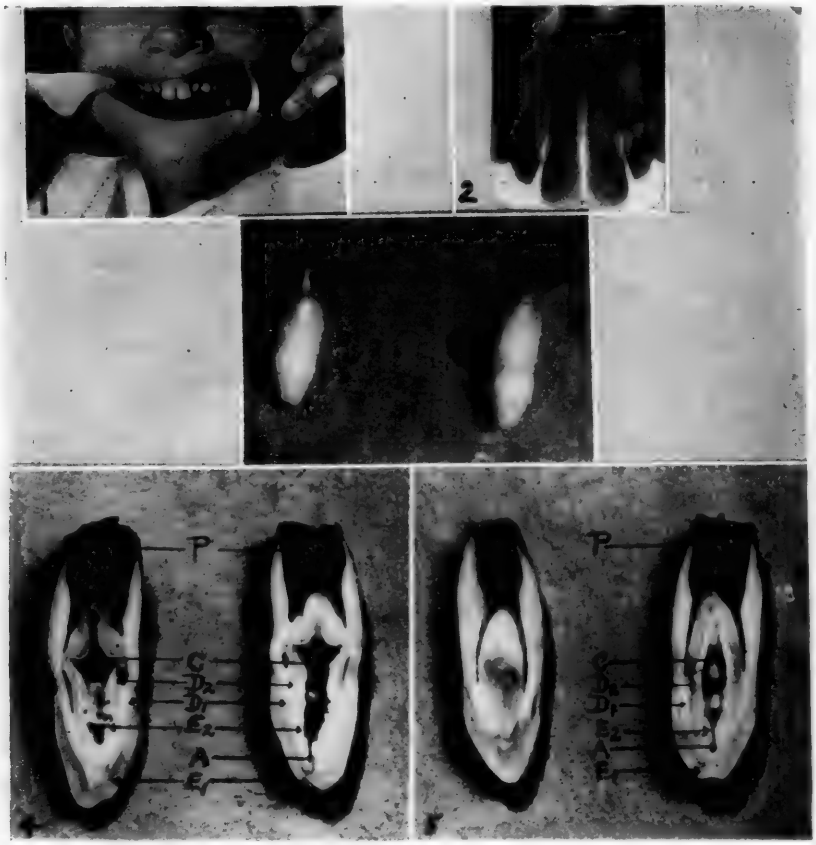

Fig. 1. Eight year old female with parulis over upper left permanent second incisor. The permanent centrals, the permanent "peg shaped" second incisors, the deciduous canines, and the deciduous molars can be seen.

Fig. 2. Radiograph showing dens in dente in each of the upper second incisors.

Fig. 3. The second incisors after removal. Granuloma attached to apical end of upper left second incisor. There was no caries in either tooth. On the incisal edge of each tooth along the flattened portion there is a minute choked up opening that leads into the cavity of the "inner tooth."

Fig. 4. Upper left second incisor longitudinally sectioned.

A—Minute opening leading into cavity C of "inner tooth."

C—Cavity of "inner tooth" containing necrotic remains of enamel organ and connective tissue of dental sacculus.

D_1—Dentin of "outer tooth."

D_2—Dentin of "inner tooth."

E_1—Enamel of "outer tooth."

E_2—Enamel of "inner tooth."

P—Pulp.

Fig. 5. Upper right second incisor longitudinally sectioned. (Legend same as in *fig. 4.*)

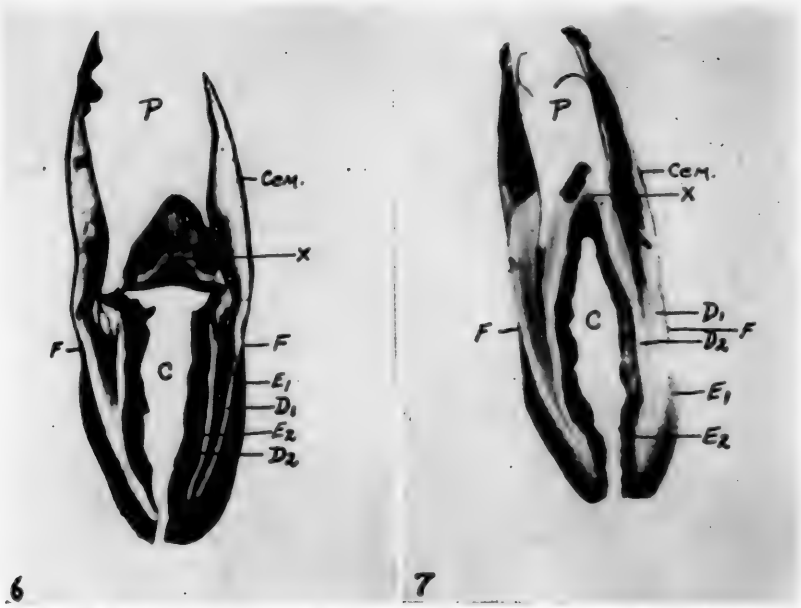

Fig. 6. Longitudinal section in mesio-distal plane of upper left second incisor

C—Cavity of "inner tooth."
Cem.—Cementum.
D_1—Dentin of "outer tooth."
D_2—Dentin of "inner tooth."
E_1—Enamel of "outer tooth."
E_2—Enamel of "inner tooth."
F—Cemento-enamel junction.
P—Pulp.
X—Apical limit of enamel of "inner tooth."

Fig. 7. Longitudinal section in mesio-distal plane of upper right second incisor. (Legend same as in *fig. 6.*)

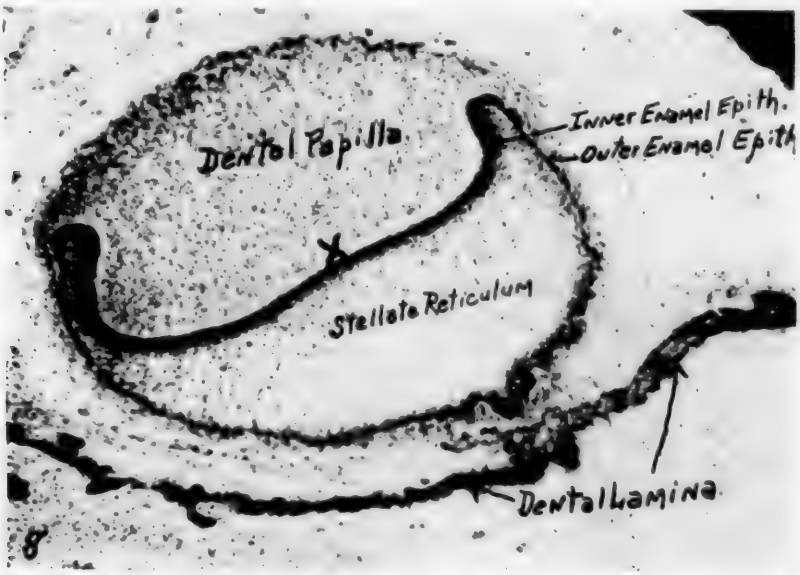

Fig. 8. Developing tooth germ of a normal upper deciduous second incisor. For a dens in dente to develop there must be a proliferation of the cells in the region "X" into the dental papilla.

rounding dental tissues continued to expand and to grow in a peripheral direction. Kitchin (2) agreed with this viewpoint. We believe that the development of a dens in dente is not a retardation in the growth of a portion of the enamel organ, but is a proliferation of the cells causing an ingrowth of the enamel organ apically into the dental papilla.

If it were a retardation in the growth of a portion of the enamel organ, then the enamel of the "inner tooth" should end at the cemento-enamel line of the "outer tooth," (*fig. 6-F* and *fig. 7-F*). However, since the enamel of the inner

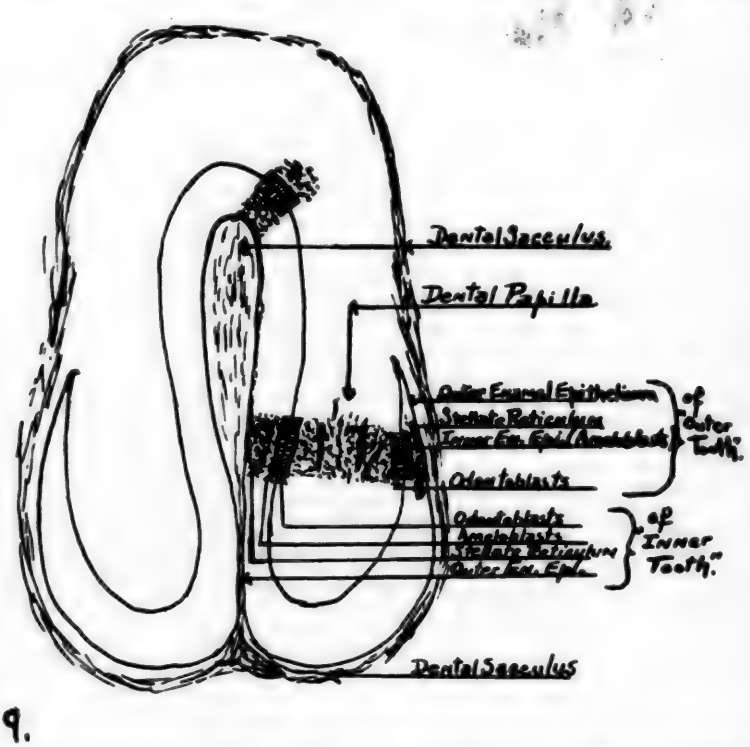

Fig. 9. Diagrammatic sketch of a tooth germ showing the way a dens in dente develops

tooth extends a considerable distance apically (fig. *6-X* and fig. *7-X*) from the cemento-enamel junction, one must conclude that there has been an active ingrowth of the cells of the enamel organ.

Fig. 8, shows the normal second incisor tooth germ of a 4 to 4½ month foetus. If at X there were a retardation in the growth of the cells of the inner enamel epithelium, we might expect a dens in dente to develop, but to develop only to the plane of the cemento-enamel junction of the "outer tooth." It would appear that the more logical explanation is that there is a definite proliferation apically of the cells in the region X (*Fig. 8*). This proliferation forms a tooth germ as

shown in *Fig. 9*, and its continued growth produces the dens in dente such as appears in *figs. 6* and *7*.

SUMMARY

The anomaly of dens in dente is a rare occurrence. This case is the only case of bilateral dens in dente reported. The case was studied by means of photographs, radiographs, and ground sections.

The authors cannot support the theory that such malformations represent retardations at one or more points of growth, but believe these malformations to be caused by a proliferation of the cells of the enamel organ into the dental papilla.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the New York Section[1]

Samuel C. Miller, *Editor, N. Y. Section*

School of Dental and Oral Surgery, Columbia University, January 30, 1947[2]

1. Psychosomatic Factors in the Etiology of Periodontal Disease. *Samuel Charles Miller and Julian M. Firestone*[3], *College of Dentistry, New York University, New York.* The war years have produced many cases of periodontal disease in both civilian and military population for which one of the important etiological factors is emotional tension. Even in necrotic gingivitis (Vincents' infection), emotional factors play a major role in causation. Since the present stress of living is developing even greater difficulties of psychologic adjustment, it is reasonable to expect that the number of such cases coming to the attention of the dentist and physician will continue to increase. Three important means by which psychomatic factors operate are discussed, (1) Emotional disturbances creating oral disease, (2) oral changes inducing mental ills, and (3) by far the greatest majority of cases are those in which the vicious circle operates with greater or smaller percentages of each factor. Eight ways in which periodontal diseases may be created by psychic disturbances are discussed, namely: (1) Reduction of local nutrition through vasospasm (2) Development of objective habits which are antagonistic to the health of the periodontal tissues, e.g. pencil biting, fingernail biting (3) By inducing excessive chewing, or grinding (4) By creating taste perversions causing the ingestion of foods which are locally and systematically harmful to periodontal health (5) By permitting insufficient food intake through limitation of gastro-intestinal function (6) By producing neglect of oral sanitation (7) By involving subjective habits which are harmful to the periodontal structures, e.g. lip biting, cheek biting, tongue thrusting (8) By causing bodily conditions inimical to the health of the periodontal tissues. The modern dental practice must include the consideration of psychosomatic factors, if it is to render a complete health service.

2. Studies in the Dietaries of Contemporary Primitive Peoples. *Meyer Klatsky, Workman's Circle, New York.* The dietaries of a number of contemporary primitive races were studied from their nutritional as well as their textural aspects. It was found that the diets of most primitive peoples are qualitatively and quantitatively deficient in nutrient value. They are monotonous, poorly balanced, and lacking in many essential elements necessary for the normal

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[3] Guest.

growth and development of a healthy body. However, despite their poor nutritive level, members of the average primitive groups studied have been found to possess large, well developed jaws and healthy teeth. We feel that this is due to the physical qualities of their diet. The foods are hard, bulky, fibrous and resistant. They stimulate function of the masticatory organs, and keep these structures in a state of health despite their unbalanced and inadequate chemical contributions. In studying dietary factors in dental abnormality and disease, more stress should be placed on the physical state and the mechanical action of foods as a functional stimulant, rather than on the chemical and nutritional aspects of the subject.

3. The Effect of the Temporal Muscle on the Form of the Mandibles. *S. L. Washburne, College of Physicians and Surgeons, Columbia University, New York.* Before any bone appears in the human fetus, the neuro-muscular system is differentiated, and the fetus is capable of movements. The bony skeleton develops under the stress of muscular contraction. While the primitive cartilagenous skeleton is mainly self-differentiating, the form of the bony skeleton depends on the balance between intrinsic factors and the stresses to which the developing bones are subjected. The rôle of the temporal muscle in forming the mandible was investigated. The temporal muscle of day-old rats was removed. Four months later the animals were sacrificed. No coronoid process was present, and the mandibular neck and condyle were smaller than normal. It is concluded that the extrinsic factors acting on the bone are more important than is usually supposed.

4. Traumatic Injuries to Children's Teeth. *E. Alan Lieban, New York.* Children leading a normal life indulge in playing and outdoor sports that often unavoidably result in accidental injuries to their teeth. There are instances where teeth are injured without visible evidence. In a short time the incident may be forgotten, yet the result of such injuries may not be evident until later. The dentist is possibly perplexed as to the best procedure to follow, and in many cases a rational diagnosis cannot be established for several months after the initial injury. The importance of frequent check-up cannot be over-emphasized. However, in the more severe types of injuries, errors in judgement frequently result in unnecessary devitalization and more often in the loss of the tooth. Prior to instituting orthodontic treatment, the elicitation of information regarding any possible injury about the mouth, is very important. The writer personally believes that in addition to complete series of roentgenograms, every tooth should be carefully pulp tested and findings recorded before any orthodontic treatment is begun. Pulpless teeth may be present without apparent discoloration or radiographic evidence and subsequently the pulp death may be attributed to orthodontics. A clinical evaluation of the various types of injuries treated, and the methods employed are presented.

5. Estrogen Implants in Female Dogs. (Preliminary Report.) *Daniel E. Ziskin, Edward V. Zegarelli and Charles Slanetz, School of Dental and Oral Surgery, Columbia University, New York.* This is a study to test the effects of estrogen implants (alpha-estradiol) in dogs. The main interest centered

about the oral tissue, although other organs were observed as well. Gross and microscopic examination were made, and complete blood counts and pertinent blood chemical analyses were included. Twenty-one females comprised our group. Thirteen dogs received implants of alpha-estradiol pellets subcutaneously. Three dogs received the estrogen implants and in addition were given 3 grams of soy bean lecithin daily incorporated into their diet. Three dogs received estrogen implants only, for several months, then 3 grams of lecithin were added to their daily diets, 28 days before the termination of the experiment in two instances and 12 days in one instance. Two controls, living under conditions identical with the rest of the group but without estrogen implants or lecithin. The average duration of the experimental procedure was 7 months. The average total dosage of alpha-estradiol over the entire period was 80 mg. All animals received implants at least twice, and some as many as 5 times. The average absorption was about 170 micrograms a day. Blood counts showed a drop in the number of red blood cells. The most consistent reaction noted was a marked drop in the number of white blood cells after an initial rise. The hemoglobin showed the least change. The skin changes consisted of alpecia, scaly dermititis, located chiefly on the exposed surfaces of the joints of the forelegs and hindlegs, somewhat resembling psoriasis in man; scabs between the toes; and scaly sores generally over the body. Gingivae revealed an inflammatory condition resembling a so-called "chronic" Vincent's infection, or, in a few, a more severe necrosis characteristic of agranulocytic angina in man. Uterus presented necrosis involving the endometrium, the myometrium or perforations. At autopsy it was found that the most severe necrosis had developed in the uterus. The gingivae were the next most seriously involved and then the vagina. The most severe blood affection seen was a marked leukopenia. Those animals receiving the soy bean lecithin were in some degree protected against the toxic symptoms. Although skin lesions developed, they came on later in the experimental period and were not as severe. The lecithin seemed to afford the most protection to the uterus.